Antti Knaapila

Genetic and Environmental Influences on Human Responses to Odors

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Department of Molecular Medicine
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and
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A C A D E M I C D I S S E R T A T I O N

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To the promoters of science and humanity

"A rose by any other name would smell as sweet"

Shakespeare, ca 1595

"A rose by any other name would not smell as sweet"

Herz and von Clef, 2001

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ABSTRACT

Olfaction, the sense of smell, has many important functions in humans. Human responses to odors show substantial individual variation. Olfactory receptor genes have been identified and other genes may also influence olfaction. However, the proportion of phenotypic variation in odor response due to genetic variation remains largely unknown. Little is also known about which genes modify specific responses to odors. This study aimed to elucidate genetic and environmental influences on human responses to odors.

Individuals from Finnish families (n=146) and Australian (n=413), British (n=163), Danish (n=336), and Finnish (n=399) twins rated intensity and pleasantness of a set of 12 (families) or 6 (twins) odors and tried to identify the odors. In addition, the participants rated their own sense of smell and annoyance experienced with different environmental odors. The odor stimuli of a commercial smell test (The Brief Smell Identification Test, B-SIT; banana, chocolate, cinnamon, gasoline, lemon, onion, paint thinner, pineapple, rose, smoke, soap, and turpentine) were presented in the family study. Based on the results of the family study and a literature survey, a new set of odor stimuli (androstenone, chocolate, cinnamon, isovaleric acid, lemon, and turpentine) was designed for the twin studies. In the family sample, heritabilities of the traits were estimated and underlying genomic regions were searched using a genome-wide linkage scan. In the pooled twin sample, variation in the measured traits was decomposed into genetic and environmental components using quantitative genetic modeling. In addition, associations between nongenetic factors (e.g., sex, age, and smoking) and olfactory-related traits were explored.

Suggestive evidence for a genetic linkage for pleasantness of cinnamon at a locus on chromosome 4q32.3 emerged from the family sample. High heritability for the pleasantness of cinnamon was found in the family but not the twin study. Heritability (additive genetic effects) of perceived intensity of androstenone odor was determined to be ~30% in the twin sample. A strong genetic correlation between perceived intensity and pleasantness of androstenone, in the absence of any environmental correlation, indicated that only the genetic correlation explained the phenotypic correlation between the traits (r=-0.27) and that the traits were influenced by an overlapping set of genes. Self-rated olfactory function appeared to

reflect the odor annoyance experienced rather than actual olfactory acuity or genetic involvement. Results from nongenetic analyses supported the speculated superiority of females' olfactory abilities, the age-related diminishing of olfactory acuity, and the influences of experience-dependent factors on odor responses.

This was the first study to estimate heritabilities and perform linkage screens for individual odors. A genetic effect was detected for only a few responses to specific odors, suggesting the predominance of environmental effects in odor perceptions.

Keywords: environmental effects, family study, genetic effects, genetic modeling, heritability, human, linkage analysis, odor, olfaction, smell, twin study

Antti Knaapila, Perintö- ja ympäristötekijöiden vaikutukset ihmisen hajuaistimuksiin
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TIIVISTELMÄ

Hajuaistilla on monia ihmiselle tärkeitä tehtäviä. Hajujen aistimisessa on ihmisten välillä huomattavaa yksilöllistä vaihtelua. Hajureseptoreita koodaavat geenit on tunnistettu ja myös muilla geeneillä saattaa olla vaikutusta hajuaistiin. Kuitenkin on vielä paljolti epäselvää, kuinka suuri osa hajujen kokemisessa esiintyvästä vaihtelusta selittyy geneettisellä vaihtelulla. Samoin tiedetään vain vähän siitä, mitkä geenit vaikuttavat kuhunkin hajuaistimukseen. Tämän tutkimuksen tavoitteena oli määrittää perintö- ja ympäristötekijöiden vaikutuksia ihmisen hajuaistimuksiin.

Suomalaisten perheiden jäsenet (n=146) sekä australialaiset (n=413), isobritannialaiset (n=163), tanskalaiset (n=336) ja suomalaiset kaksoset (n=399), arvioivat 12 (perheet) tai 6 (kaksoset) hajun voimakkuuden ja miellyttävyyden sekä yrittivät tunnistaa hajut. Lisäksi osallistujat arvioivat oman hajuaistinsa toiminnan ja ympäristön hajujen häiritsevyyden. Perhetutkimuksessa käytettiin kaupallisen hajutestin hajuja (The Brief Smell Identification Test, B-SIT; banaani, suklaa, kaneli, bensiini, sitruuna, sipuli, tinneri, ananas, ruusu, savu, saippua ja tärpätti). Perhetutkimuksen tulosten, kirjallisuustutkimuksen ja muitten näkökohtien pohjalta suunniteltiin uusi hajuärsykesarja (androstenoni, suklaa, kaneli, isovaleriaanahappo, sitruuna ja tärpätti) käytettäväksi kaksostutkimuksissa. Perheaineistosta laskettiin ominaisuuksien periytyvyysasteet ja etsittiin ominaisuuksiin kytkeytyneitä perimän alueita koko genomin laajuisella seulonnalla. Kaksosaineistot yhdistettin ja mitattujen ominaisuuksien vaihtelu jaettiin geneettiseen ja ympäristön aiheuttamaan osaan käyttäen kvantitatiivista geneettistä mallitusta. Lisäksi tutkittiin ei-geneettisten tekijöiden (kuten sukupuoli, ikä ja tupakointi) yhteyksiä hajuaistiin liittyviin ominaisuuksiin.

Perheaineistossa saatiin suuntaa-antavaa todistusta kanelin miellyttävyyden kytkeytymisestä geenialueeseen kromosomissa 4q32.3. Kanelin miellyttävyydelle havaittiin korkea periytyvyysaste perhetutkimuksessa, mutta ei kaksos-tutkimuksessa. Androstenonin hajun koetun voimakkuuden periytyvyysasteeksi (additiiviset geneettiset vaikutukset) määritettiin kaksosaineistossa ~30%. Voimakas geneettinen korrelaatio androstenonin koetun voimakkuuden ja miellyttävyyden välillä, ympäristöllisen korrelaation puuttuessa, viittaa siihen, että geneettinen korrelaatio voi yksin selittää ominaisuuksien välisen fenotyyppisen korrelaation

(r=-0.27) ja että ominaisuuksien taustalla vaikuttaa samoja geenejä. Hajuaistin toiminnan omat arviot näyttivät heijastavan enemminkin hajujen koettua häiritsevyyttä kuin varsinaista haistamiskykyä tai geneettistä vaikutusta. Ei-geneettisten analyysien tulokset tukivat käsityksiä naisten paremmasta haistamiskyvystä, ikään liittyvästä hajuaistin heikkenemisestä sekä kokemusperäisistä vaikutuksista hajujen kokemiseen.

Tämä oli ensimmäinen tutkimus, jossa määritettiin yksittäisten hajujen aistimisen periytyvyysasteita ja suoritettiin koko perimän laajuinen hajuaistimuksiin kytkeytyneiden alueiden etsintä. Geneettinen vaikutus havaittiin vain harvojen yksittäisten hajujen kohdalla, mikä kertoo ympäristötekijöiden hallitsevuudesta hajujen aistimisessa.

Avainsanat: ympäristön vaikutus, perhetutkimus, geneettinen vaikutus, geneettinen mallitus, periytyvyysaste, ihminen, kytkentäanalyysi, tuoksu, hajuaisti, haju, kaksostutkimus

CONTENTS

ABBREVIATIONS

A	additive genetic effects
a^2	proportion of variance due to additive genetic effects
bp	base pair
B-SIT	Brief Smell Identification Test™
C	shared environmental effects
cM	centimorgan
CNV	copy number variation
D	dominant genetic effects
DNA	deoxyribonucleic acid
DZ	dizygous
E	nonshared environmental effects
e^2	proportion of variance due to nonshared environmental effects
EC1-EC3	extracellular loops 1-3 (of OR)
GPCR	G-protein-coupled receptor
csGPCR	chemosensory GPCR
G_{olf}	olfactory-specific G-protein
IC1-IC3	intracellular loops 1-3 (of OR)
kb	kilobase
LOD	logarithm of odds
MRCA	most recent common ancestor
MZ	monozygous
OB	olfactory bulb
OBP	odorant binding protein
OE	olfactory epithelium
OR	olfactory receptor (=odorant receptor)
OSN	olfactory sensory neuron

r	Pearson correlation coefficient
r_{MZ}	intraclass correlation within MZ twin pairs
r_{DZ}	intraclass correlation within DZ twin pairs
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
SPG	segregating pseudogene
TM1-TM7	transmembrane domains 1-7 (of OR)
UPSIT	University of Pennsylvania Smell Identification Test
URTI	upper respiratory track infection
VNO	vomeronasal organ
θ	recombinant fraction

In addition, standard one-letter abbreviations for amino acids and nucleotides are used.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals (I-IV):

I Knaapila, A., Keskitalo, K., Kallela, M., Wessman, M., Sammalisto, S., Hiekkalinna, T., Palotie, A., Peltonen, L., Tuorila, H., and Perola, M. 2007. Genetic component of identification, intensity and pleasantness of odours: a Finnish family study. *European Journal of Human Genetics* 15(5): 596-602.

II Knaapila, A., Tuorila, H., Silventoinen, K., Wright, M.J., Kyvik, K.O., Cherkas, L.F., Keskitalo, K., Hansen, J., Martin, N.G., Spector, T.D., Kaprio, J., and Perola, M. 2008. Genetic and environmental contributions to perceived intensity and pleasantness of androstenone odor: an international twin study. *Chemosensory Perception* 1(1): 34-42.

III Knaapila, A., Tuorila, H., Silventoinen, K., Wright, M.J., Kyvik, K.O., Keskitalo, K., Hansen, J., Kaprio, J., and Perola, M. Environmental effects exceed genetic effects on perceived intensity and pleasantness of several odors: a three-population twin study. *Behavior Genetics, in press.*

IV Knaapila, A., Tuorila, H., Kyvik, K.O., Wright, M.J., Keskitalo, K., Hansen, J., Kaprio, J., Perola, M., and Silventoinen, K. Self-ratings of olfactory function reflect odor annoyance rather than olfactory acuity. *Laryngoscope, accepted for publication.*

In addition, some unpublished material is presented.

1 INTRODUCTION

Olfaction, the sense of smell, has many fascinating features. Odors evoke associations, emotions, and vivid memories from as early as childhood (Willander and Larsson, 2006). Spoiled food and many other potential dangers, such as fire and harmful chemicals, are often detected based on their smell (Doty, 2007a). Together with tastes, odors are central for the flavor of foods and beverages, thus contributing to food choice (Shepherd, 2006). Odors are also involved in social interactions in conscious and perhaps also unconscious ways (Stockhorst and Pietrowsky, 2004; Wysocki and Preti, 2004). Although olfaction is not vital for humans, its importance for quality of life is indisputable (Hummel and Nordin, 2005).

The discovery of the gene family encoding olfactory receptors in the early 1990s (Buck and Axel, 1991) provided impetus for research in the genetics of olfaction. Consequently, individual olfactory receptor genes have been identified (Niimura and Nei, 2003; Malnic et al., 2004) and many intriguing characteristics of the gene family exposed. The discoverers of the gene family, Linda Buck and Richard Axel, were awarded the Nobel Prize for their achievements in 2004.

The olfactory receptor gene family with its ~850 genes forms one of the largest gene families in humans. However, over half of these genes appear to be nonfunctional pseudogenes (Niimura and Nei, 2003). Interestingly, some of these genes display both functional and nonfunctional variants (Menashe et al., 2003), contributing substantially to genetic variation and making it difficult to determine the exact number of the genes. Characteristic features of these genes also include their distribution over almost all chromosomes (Glusman et al., 2001) and abundant copy number variation (Nozawa et al., 2007). In addition, humans have less olfactory receptor genes than most other mammals (Niimura and Nei, 2007). Taken together, these findings indicate a dynamic evolutionary history for this gene family.

The relative contribution of genetic variation to phenotypic variation in responses to odors is largely unknown. Moreover, the first associations of polymorphisms in olfactory receptor genes with psychophysically measured odor perceptions were reported only recently (Keller et al., 2007; Menashe et al., 2007). Recent methodological advancements will facilitate the discovery of expression patterns of olfactory receptor genes (Zhang et al., 2007) and ligand specificity of the corresponding receptors (Touhara, 2007). However, exploring genetics related to overt human responses to odors requires time-consuming psychophysical testing.

2 REVIEW OF THE LITERATURE

This review focuses on the genetics of olfaction in humans; other species are referred to only for comparison. The olfactory system, measures for responses to odors, and nongenetic factors associated with these responses are introduced. After reviewing the genetics of olfaction, some methods for exploring it are discussed.

2.1 Olfactory system

The human olfactory system can discriminate thousands of odors, but an accurate number is difficult to pinpoint (Firestein, 2001). The olfactory system is responsible for the odor perception and discrimination process. It includes two main phases: peripheral detection of odorants in nasal cavities and central processing of the olfactory signal in the brain.

2.1.1 Anatomical overview

Peripheral detection of odorants occurs in the olfactory epithelium (OE). The OE is located in olfactory clefts in the roof of the nasal cavities, just below and between the eyes. The nasal cavities are separated, and thus, olfaction is bilateral. Both the left and right cavities have an OE of about 2.5 cm^2. Odorants can enter the OE in two ways: via nostrils (orthonasal route) and via the nasopharynx (retronasal route) (**Figure 1**).

The olfactory signal is transmitted from the OE to the olfactory bulb (OB) through the cribriform plate, a sieve-like structure. From the OB, the signal is further transmitted to the olfactory cortex of the brain. The olfactory signal is transmitted through cranial nerve I (olfactory nerve), the first of the twelve cranial nerves. It is remarkable that in the brain, the olfactory tract does not go through the thalamus (like other sensory information), but is closely associated with the limbic system (Stockhorst and Pietrowsky, 2004).

In addition to the olfactory system, most odorants, at least at high concentrations, also activate the trigeminal system. The trigeminal system provides innervation to the nasal cavities for the detection of such somatosensory signals as pain, touch, and irritation (Landis et al., 2005). The trigeminal signal is transmitted through cranial nerve V (Doty, 2001). The olfactory and trigeminal systems are closely related anatomically and physiologically, and thus, a strong interaction exists between the systems (Landis et al., 2005). However, the focus here is on the olfactory system.

The vomeronasal organ (VNO) contributes to the detection of pheromones in some species, e.g., the mouse. Strictly, the VNO is considered to be part of the vomeronasal system (the accessory olfactory system), which is distinct from the main olfactory system (Doty, 2001). A large body of evidence (reviewed by Baxi et al., 2006) suggests that these two systems have overlapping functions in several species; the vomeronasal system is not equivalent to a pheromone detecting system.

Detection of pheromones using the VNO is very unlikely in humans (reviewed by Wysocki and Preti, 2004). First, the VNO, or the vomeronasal duct, does not exist in all individuals. The human VNO appears to be vestigial and is likely to disappear before birth (Firestein, 2001). Second, the VNO, when present, appears to be nonfunctional. However, some pheromone-like responses may be mediated by the (main) olfactory system (Wysocki and Preti, 2004; Landis et al., 2005).

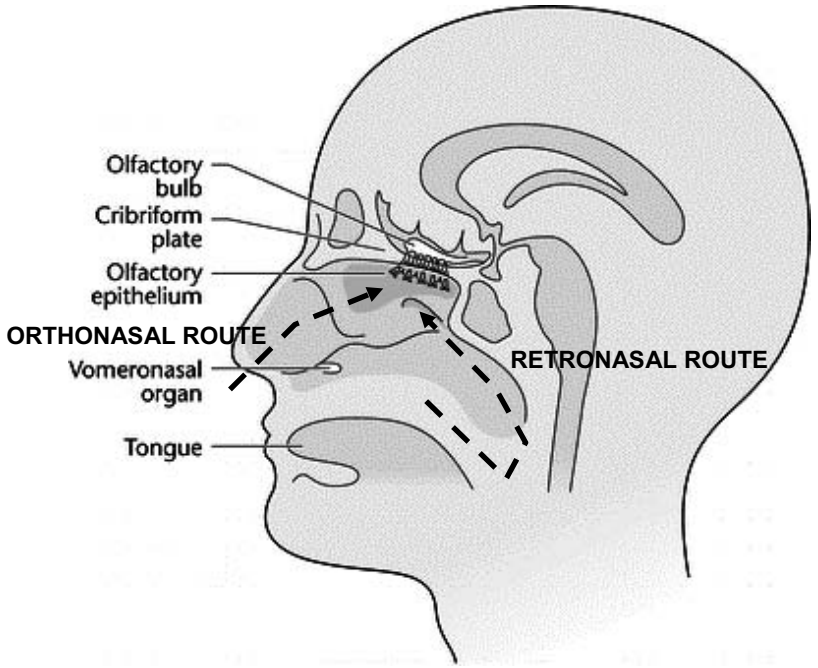

Figure 1. ***Schematic presentation of the organization of the olfactory system (modified from Spielman et al. Chemosensory Systems. In: Encyclopedia of life sciences. John Wiley & Sons, Ltd: Chichester. http://www.els.net/).***

2.1.2 Olfactory receptors

Olfactory receptors (odorant receptors, ORs) are members of a larger group of receptors, the G-protein-coupled receptors (GPCRs). The G-protein refers to a guanyl nucleotide binding protein. Mammalian GPCRs have been classified based on shared sequence motifs. ORs belong to class A GPCRs, which are also called rhodopsin-like GPCRs after the prototype of the class, rhodopsin. The GPCRs can also be grouped based on their function. In this case, ORs are included in chemosensory GPCRs (csGPCRs). ORs form the largest family of csGPCRs. Taste and VNO receptors are also included in csGPCRs (Zarzo, 2007).

ORs, like all GPCRs, have a characteristic structure of seven transmembrane domains (TM1-TM7) composed mostly of hydrophobic amino acid residues and predicted to form α-helices. These seven hydrophobic TMs are connected by three intracellular (IC1-IC3) and three extracellular loops (EC1-EC3), formed of mostly hydrophilic residues (Breer, 2003; Zarzo, 2007). The GPCRs have an extracellular amino-terminus (N-terminus) and an intracellular carboxy-terminus (C-terminus). The N- and C-termini of ORs are short, only 20-30 amino acids (Zozulya et al., 2001). The N-termini of ORs include a consensus sequence for N-glycosylation sites. The C-termini comprise putative phosphorylation sites, likely to be phosphorylated by second messenger-activated kinases and receptor kinases for uncoupling the transduction cascade (Breer, 2003). The ORs are located in the olfactory sensory neurons (OSNs): the TMs are buried in the lipid bilayer membrane of the OSNs and the loops reside in the water phase (**Figure 2**).

The amino acid chain of most ORs comprises 300-350 residues (Glusman et al., 2001). According to Fuchs et al. (2001), the length of the OR protein is 313±8 amino acid residues and is highly invariable, implying that functional constraints may prohibit larger variation.

The ORs include seven conserved cysteines, of which two are common to all GPCRs and the other five are unique to the ORs. The intracellular half of the OR protein is more positively charged and more conserved than the extracellular half (Fuchs et al., 2001). At least some ORs are metalloproteins. EC2 has a putative metal binding site: a sequence motif HXXCD may form a complex with Zn(II) or Cu(II) ions. This could explain why good ligands for metal ion coordination complexes, such as thiols, amines, nitriles, and isonitriles, are very strong-smelling odorants (Breer, 2003; Zarzo, 2007).

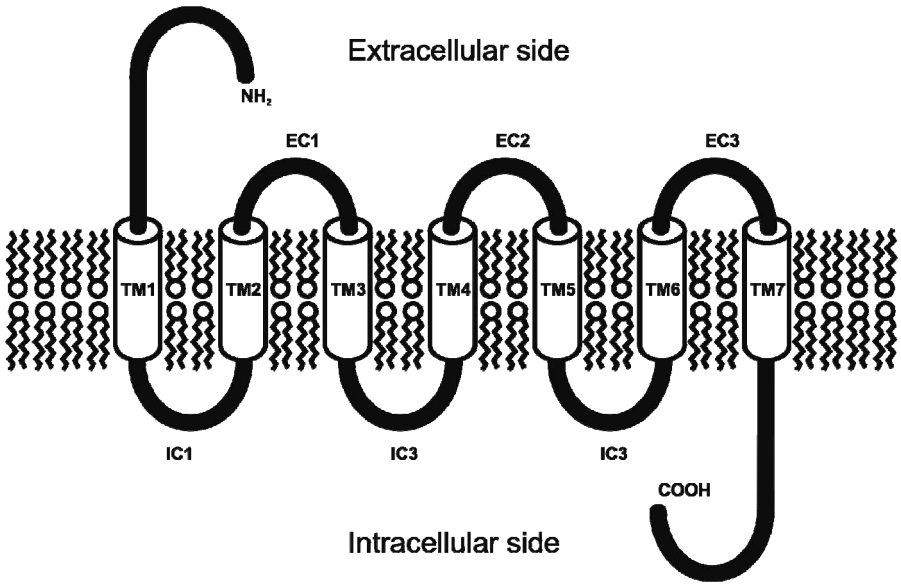

Figure 2. ***Schematic presentation of the structure of an olfactory receptor. Seven helical hydrophobic transmembrane domains (TM1-TM7) of the receptor are embedded in the lipid bilayer membrane of an olfactory sensory neuron and connected to three extracellular (EC1-EC3) and three intracellular (IC1-IC3) hydrophilic loops residing in the water phase.***

According to Buck and Axel (1991), the rat ORs share 40-80% amino acid identity. The study of Glusman et al. (2001) indicated that the 40% cut-off in protein identity also powerfully discriminates between members of the human OR superfamily and the other GPCRs. Fuchs et al. (2001) found minimum pairwise similarities of 20-25% identity in human ORs, but even this low similarity was sufficient to distinguish the ORs from the other GPCRs.

The ORs include both highly conserved and highly divergent structures. Strong sequence conservation occurs in the intracellular loops (Zozulya et al., 2001). The junction of the TM3 and IC2 includes the DRY motif, which is conserved in all GPCRs (Breer, 2003). It is included in the MAYDRYVAIC motif, which is one of the 4-5 consensus sequences frequently used as criteria for recognizing the ORs in data mining studies (Zozulya et al., 2001; Malnic et al., 2004). Other highly conserved regions include the TM1, TM2, and TM7 (Zozulya et al., 2001). In contrast, structural diversity is highest in extracellular loops EC1 and EC3 (Zozulya et al., 2001) and in the central transmembrane domains TM3, TM4, and TM5 (Buck and Axel, 1991). These transmembrane domains include hypervariable regions that

are likely to form ligand-binding sites for odorants (Fuchs et al., 2001). The relative variability of various parts and residues of the ORs is extensively reviewed by Fuchs et al. (2001) and Zozulya et al. (2001).

2.1.3 Physiology of odor perception

The term odor should be distinguished from the term odorant. Odorants are chemical substances, usually small volatile molecules. Odor may emerge from a single type of molecule, but also from a mixture of a large number of different odorants. Odor is a percept; it is the product of a plastic nervous system, and thus, the perception of odors can be modified by exposure and learning (Stockhorst and Pietrowsky, 2004).

Peripheral detection of odorants

The ORs are responsible for odorant detection in the OE. Individual OSNs express only one type of OR gene each (Malnic et al., 1999). The ORs are embedded in the membrane of 5-10 immotile cilia of each OSN. The OE is covered with a mucus layer, which odorants have to pass to enter the binding sites of the ORs (**Figure 3**). Odorant binding proteins (OBPs) are suggested to help hydrophobic odorants to traverse the aqueous milieu of the mucus layer. Different types of OBPs have been found to have specific ligand-binding profiles, indicating that OBPs may be active filters rather than passive shuttles for odorants (Breer, 2003).

Ligand specificity of olfactory receptors is not always very strict. One receptor type can recognize multiple (likely structurally similar) odorants. One odorant, in turn, is recognized by multiple receptor types (likely from the same OR subfamily), however, with varying affinities. This implies that the olfactory system uses some kind of combinatorial coding to discriminate the vast array of odorants (Breer, 2003). Some ORs appear to be finely tuned, recognizing only a very restricted set of odorants, while other ORs are broadly tuned, detecting a wide repertoire of molecules (Zarzo, 2007).

Binding of an odorant molecule to OR initiates a signal transduction cascade in the OSN cell. First, the odorant-bound OR activates the olfactory-specific G-protein (G_{olf}). According to Zarzo (2007), the conformational changes of the OR during odorant binding are not known in detail, and also details of the G-protein activation are unclear. However, after activation, the G_{olf} activates the adenylyl cyclase type III, which catalyzes the conversion of abundant intracellular adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). This second messenger opens a nonselective cation channel, a cyclic nucleotide-gated channel, which allows Na^+ and Ca^{2+} ions to flow into the cell. Ca^{2+} ions activate also a channel for outward flow of Cl^- ions. This transduction cascade leads to the generation of an action

potential, which sends the olfactory signal via the axons of the OSN to the OB (**Figure 3**) (Firestein, 2001; Ache and Young, 2005; Ma, 2007).

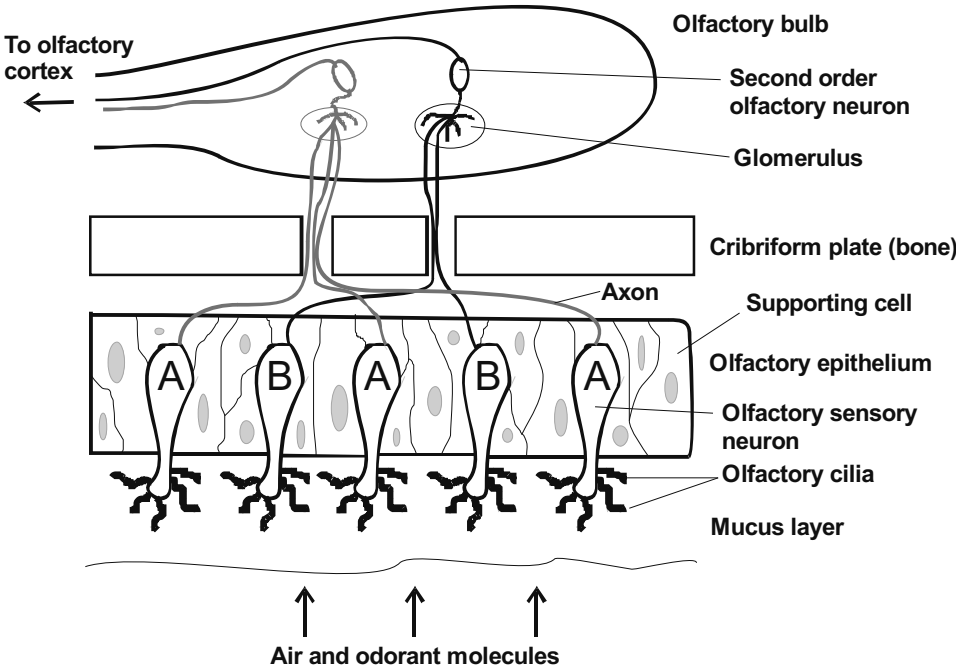

Figure 3. ***Schematical presentation of the olfactory epithelium and olfactory bulb. Airborne odorants have to traverse mucus layer to reach olfactory receptors in the cilia of olfactory sensory neurons. Olfactory sensory neuron populations (two are depicted here, denoted A and B) express only one type of olfactory receptor gene each. Olfactory signals elicited by the interaction of odorants with olfactory receptors are transferred from olfactory sensory neurons to olfactory bulb, where the signals from the same population of neurons converge in glomerulus (modified from Firestein, 2001, and Zarzo, 2007).***

Central processing of olfactory signal

The olfactory signal is transmitted from the OSN through the cribriform plate to the OB, the first cortical olfactory structure in the brain. Here, the signal synapses with the second-order neurons, the mitral and tufted cells. These neurons form spherical units called glomeruli. Each glomerulus connects signals from OSNs expressing the same type of OR only. Also, each OSN sends its signal to only a single glomerulus

(on lateral and medial side of the OB). Thus, olfactory signals from specific OSNs are efficiently converged in the OB (**Figure 3**) (Zelano and Sobel, 2005). As every odorant is recognized by several OR types with varying affinities, detection of odorants leads to an odor-specific activation pattern at the glomerular level. This is the basis for olfactory coding, which is responsible for discrimination between different odorants (Landis et al., 2005).

The principle of combinatorial coding suggests that similar odors are detected by different but overlapping sets of ORs (Malnic et al., 1999). Almost infinite combinations of OR responses to odorants probably account for the capacity of the olfactory system to discriminate numerous odors. Also, it explains why small changes in the structure of an odorant can produce dramatic changes in its perceived odor (Breer, 2003). However, predicting the odor of a molecule based solely on its physicochemical properties remains difficult (Landis et al., 2005).

2.1.4 Measures for responses to odors

Responses to odors can be measured using psychophysical, electrophysiological, and imaging techniques; this review focuses on the first one. In psychophysical methods, an individual gives a conscious response to the presented stimulus, by, for instance, rating the intensity or pleasantness of the odor, by indicating whether or not any odor is detected, or by choosing the most appropriate descriptor for the odor from the alternatives provided (Doty and Laing, 2003). Electrophysiological techniques are based on the recordings of objective electric responses, such as olfactory event-related potentials (Hummel and Kobal, 2002) and electro-olfactograms (Knecht and Hummel, 2004), elicited by the presented odor stimuli. Imaging methods include functional magnetic resonance imaging, positron emission tomography, and magnetic source imaging (Landis et al., 2005). Although psychophysical methods are commonly referred to as subjective and electrophysiological and imaging techniques as objective test methods (Simmen and Briner, 2006), this dichotomy may be misleading (Doty, 2007a). Electrophysiological and imaging methods are used mostly in scientific research, but psychophysical methods are used widely also in clinical assessments (Simmen and Briner, 2006; Doty, 2007a).

The odor stimuli can be presented using various methods. An olfactometer is an apparatus for accurate and controlled delivery of odorant(s) via its pipes connected to the subject's nasal cavities. Odorants are also frequently presented as sniffed from the head space of a bottle (e.g. glass vial or squeeze bottle) containing the odorant in solution. Odorants can also be microencapsulated in odor labels (odor strips), from

which the odorants are released for evaluation by scratching the surface (Doty and Laing, 2003).

Several classes of responses to odors can be measured using psychophysical methods: odor detection (sensitivity), recognition (of odor quality), identification (naming), discrimination, perceived intensity (suprathreshold scaling), pleasantness, familiarity, annoyance, and memory. Of these, measurements of responses related to olfactory function and odor hedonics are discussed below.

Olfactory function

Psychophysically measured responses to odor stimuli are commonly used to measure overall olfactory function (olfactory acuity). The olfactory function is most frequently measured using tests based on odor identification tasks or determination of detection thresholds of standard odorant(s) (Doty, 2007a). The ability to discriminate between similar odors has also been used to measure the olfactory function (Eibenstein et al., 2005). In addition, ratings of perceived intensity of odorants at suprathreshold concentrations (readily detectable by individuals with a normal sense of smell) are frequently used. However, this method has been argued to be less sensitive to olfactory dysfunction than the detection threshold and odor identification tests (Doty and Laing, 2003).

The detection threshold (absolute threshold, olfactory threshold) is the lowest concentration of an odorant where its presence is reliably detected. At this concentration, usually only a faint presence of something is detected, but the quality of the odor cannot be judged. The lowest concentration where the odor quality is reliably discerned is called recognition threshold. The detection threshold values appear to be more reliable than the recognition thresholds (Doty et al., 1995). Standard odorants for which detection thresholds are typically used to measure olfactory function include *n*-butanol (dissolved in water) and phenyl ethyl alcohol (dissolved in mineral oil). The thresholds are usually determined by presenting consecutive sets of two or more stimuli (one with the odorant and one or more blanks) to the subject and asking him/her to choose the odorous one (or the one with the target quality). Such forced-choice procedures are typically more reliable, produce lower threshold values, and are less susceptible to contamination by response bias (conservatism/liberalism in reporting the presence of an odor) than the nonforced choice procedures in which each stimulus is judged as odorous or nonodorous (Doty and Laing, 2003). The thresholds can be determined simply by presenting odorants from low to high concentration (ascending methods of limits) or by calculating the mean after several reversals around the threshold (single-staircase method) (Doty and Laing, 2003; Keller and Vosshall, 2004). The latter method appears to be more reliable (Doty et al., 1995).

Odor identification procedures generally consist of a set of odorant stimuli that is readily detected by individuals with a normal sense of smell. The subject's task is typically to indicate whether or not the given quality descriptor corresponds to the odor stimulus (yes/no identification), to name the odor without any given response alternatives, or to choose the most appropriate descriptor for each stimulus from a limited set of alternatives provided (multiple-choice identification). The last one is the most popular because free naming of even familiar odors is often difficult and the chance rate of a yes/no identification (50%) is considerably higher than in typical four-alternative multiple-choice identification tests (25%), which have a greater statistical power (Doty and Laing, 2003). The stimuli are also usually easy to identify with the help of the alternatives provided. Thus, most odor identification tests are designed to reveal olfactory deficits (Simmen and Briner, 2006), not to discriminate between normal and excellent olfactory acuity. In a clinical context, failure to identify an odor indicates impaired detection (or recognition) performance. The lower the score of correct identifications, the lower the olfactory function is thought to be. However, identification of an odor is based not only on the perception of the odor but also on cognitive factors. An individual may perceive an odor clearly (indicative of normal sense of smell) but misidentify the odor due to its unfamiliarity. This inflates the results of odor identification tests, especially when they are used in incompatible cultural settings (Eibenstein et al., 2005). Nonetheless, Doty and Laing (2003) argued that clinical assessment of olfactory function can be made using only simple stimulus presentation equipment as long as the stimuli are presented in a reliable manner and normative data are available for evaluating whether a patient's performance is normal or impaired.

The most frequently used odor identification test is probably the University of Pennsylvania Odor Identification Test (UPSIT; Doty et al., 1984a). It comprises the identification of 40 scratch-and-sniff odor stimuli, each of which is named from four alternatives. Since UPSIT includes some odors specifically familiar to the American culture (test developed in the USA), a shortened version of the test, the Brief Smell Identification Test (B-SIT, formerly known as Cross-Cultural Smell Identification Test, CC-SIT; Doty et al., 1996), was designed. The B-SIT contains 12 scratch-and-sniff odor stimuli derived from the UPSIT. The Scandinavian Odor Identification Test (Nordin et al., 1998) is comprised of 16 odors familiar to Scandinavian populations. Similarly, the European Test of Olfactory Capabilities (Thomas-Danguin et al., 2003) includes an identification task of 16 odors familiar to European cultures. Another European odor identification test (with 16 odorant stimuli) is included in the Sniffin' Sticks test set together with the odor discrimination and detection threshold tests (Kobal et al., 1996). Individual psychophysical test kits for measuring olfactory function are more extensively reviewed by Eibenstein et al. (2005), Koskinen (2005), and Doty (2007a).

Odor hedonics

The most immediate response to an odor is whether it is liked or not. An individual can normally easily say whether he/she likes a perceived odor even when the odor cannot be identified. From this perspective, measurement of odor hedonics is very promising. However, the degree of pleasantness is always subjective. Indeed, it has been argued that no pure odorant stimulus (eliciting no trigeminal sensation) can universally be deemed to be pleasant or unpleasant (Herz, 2001). The pleasantness of an odor is influenced by associations that emerge during the odor perception (Distel and Hudson, 2001). These associations can be modified by cognitive inputs, such as labeling (Bensafi et al., 2007). Pleasantness and familiarity of an odor are usually highly correlated; familiar odors are liked more than unfamiliar ones (Ayabe-Kanamura et al., 1998; Distel et al., 1999). Thus, while it is easy to gather evaluations of pleasantness of odors, the subjective and context-dependent nature of these ratings make it difficult to estimate the absolute pleasantness of odors. Nonetheless, measuring the pleasantness of odors of products and understanding factors that affect this response have a high practical value in the food, wine, and fragrance industries.

2.1.5 Individual variation in responses to odors

More variation has been hypothesized to be present in olfactory abilities than in any other sense (Zhang and Firestein, 2007). This variation is reflected in olfactory dysfunctions and normal physiological variation such as specific anosmias. After introducing these concepts, the most salient nongenetic factors associated with olfactory performance are reviewed.

Olfactory dysfunction

Individuals with normal olfactory acuity are referred to as normosmics (or osmics). Hyposmics have an impaired sense of smell (hyposmia, also called microsmia), and anosmics suffer from a total absence of olfactory abilities (anosmia, “smell blindness”). Brämerson et al. (2004) reported that prevalences of anosmia and hyposmia were 5.8% and 13.3%, respectively, in a representative Swedish sample (n=1387; aged ≥20 years). Similarly, Landis et al. (2004) reported proportions of anosmics and hyposmics to be 4.7% and 16%, respectively, in a representative German sample (n=1240; aged 5-86 years). These studies were based on results of odor identification tests. However, chronic smell problems were self-reported by only 1.4% of about 80000 noninstitutionalized adult respondents in the United States in 1994 (Hoffman et al., 1998). This discrepancy may be explained by individuals' low awareness of their smell deficits, as earlier suggested by several studies (Nordin et al., 1995; Murphy et al., 2002; Gudziol et al., 2007).

Olfactory deficits can be innate (e.g. congenital general anosmia) or acquired (e.g. anosmia due to an injured olfactory nerve after a blow to the head). Olfactory impairments can be permanent or transient (e.g. infections and allergies related to nasal inflammations). An olfactory dysfunction can also be classified as to whether it is a symptom of a disease (syndromic anosmia) or not (isolated anosmia). In addition to quantitative olfactory disorders (hyposmia and anosmia), there are qualitative smell disorders (dysosmias). Parosmia refers to an impaired perception of odor qualities, e.g. experiencing odors incorrectly as unpleasant. Phantosmia denotes odor hallucinations, i.e. odor perceptions in the absence of odor stimuli (Jones and Rog, 1998; Simmen and Briner, 2006). Landis et al. (2004) found the prevalence of parosmia and phantosmia to be ~2% and ~1%, respectively. The origins of parosmia and phantosmia are unknown, but they often occur concomitantly with quantitative smell disorders (Landis et al., 2004).

Specific anosmia

Odorant-specific impairments of olfactory performance exist in addition to the aforementioned (general) anosmia and hyposmia. A decreased, if not totally absent, ability to perceive the odor of a specific odorant when an individual's sense of smell is otherwise normal is called specific anosmia (also referred to as selective anosmia). Whissell-Buechy and Amoore (1973) defined an individual as specifically anosmic to a studied odorant if his/her detection threshold for that odorant was over two standard deviations beyond the mean value. Although an odorant-specific impairment may not be total anosmia (but rather specific hyposmia), the term specific anosmia is widely used and is employed in this text, too. Specific anosmia has been suggested to be due to heriditary defects in OR genes (Breer, 2003).

Age

Olfactory abilities decline with increasing age (Rawson, 2006). Odor identification scores were found to decrease consistently after the age of fifty in large studies exploring the general populations (Doty et al., 1984b [n=1955]; Wysocki and Gilbert, 1989 [n=1.4 million, the *National Geographic* Smell Survey]; Murphy et al., 2002 [n=2491]) and the healthy elderly (Larsson et al., 2004 [n=1906]). The results of these cross-sectional studies are supported by a three-year longitudinal study by Ship et al. (1996). Absolute sensitivity to odors (as measured using detection thresholds) appears to decline slowly long before old age (Cain and Stevens, 1989; Cowart, 1989). Suprathreshold intensities of odors are also rated as lower by older individuals than their younger peers (Murphy, 1993). Age is argued to have a stronger effect on olfactory abilities than either sex or smoking (Doty, 1997).

The severity of the age-related olfactory decline is, however, highly variable among individuals. Odor identification scores exhibit more variation in the elderly than in

young adults (Doty et al., 1984b; Koskinen et al., 2003). Thus, elderly individuals can also have a relatively high olfactory acuity, although anosmia is indisputably more frequent among the elderly. A group of centenarians (mean age 105 years) with relative good health showed far better olfactory abilities than anticipated (Elsner, 2001). Elsner (2001) interpreted this as support for the view that age-related diseases (such as Alzheimer's disease), not age itself, are the causal factors for accelerated olfactory decline in old age, and thus, olfactory testing would be an appropriate addition to diagnostic tests for such diseases. However, a slow, gradual fading of olfactory functioning can also occur in healthy individuals (Cain and Stevens, 1989; Ship et al., 1996). Aging appears to disrupt the sense of smell more effectively than the sense of taste (Stevens et al., 1984; Stevens and Cain, 1985; Cowart, 1989; Murphy, 1993). The elderly also report chronic smell problems more frequently than chronic taste problems (Hoffman et al., 1998).

The prevalence of olfactory loss in the elderly is marked. In the general population, Doty et al. (1984b) observed that more than 60% of subjects aged 65-80 years had a major olfactory impairment, with nearly one-fourth being anosmic. Similarly, Murphy et al. (2002) estimated the prevalence of olfactory impairment to be about 6%, 17%, 29%, and 63% among individuals aged 53-59, 60-69, 70-79, and 80-97 years, respectively. Among a group of healthy elderly persons (aged 56-88 years), an impaired sense of smell was found in 55% of individuals but only 6% were anosmic (Nordin et al., 1995).

The decline in olfactory abilities proceeds in a trait-specific and odorant-specific manner. Detection thresholds tend to increase (indicating decline in sensitivity) linearly after adolescence (Cain and Stevens, 1989; Cowart, 1989). According to the *National Geographic* Smell Survey (Wysocki and Gilbert, 1989), the ability to detect odorants (at suprathreshold concentration) is normally stable during adulthood, until it starts to decrease around the age of 40-60 years, depending on the odorant. Likewise, declines in odor identification ability and ratings for odor intensities were odorant-specific. Intensity ratings for odors tend to be highest in adolescents, decreasing consistently with age. By contrast, odor identification ability appears to form inverse U-shaped relationships with age as young adults or the middle-aged outperform adolescents and elderly individuals. A similar curvilinear relationship between odor identification score and age was found by Doty et al. (1984b). In addition, children are poorer at odor discrimination than adults (Stevenson et al., 2007), but the ability to discriminate odors decreases again in old age (Kaneda et al., 2000). In conclusion, it appears that olfactory sensitivity peaks at a very young age, while traits also requiring cognitive abilities, such as the ability to identify odors, peak later.

Sex

Females have superior olfactory abilities to males (Brand and Millot, 2001). Females outperform males in odor identification in all (or nearly all) age groups (Doty et al., 1984b; Wysocki and Gilbert, 1989; Murphy et al., 2002; Larsson et al., 2004). However, a significant sex difference in odor identification has not been found in all studies (e.g. Ship et al., 1996; Larsson et al., 2000). Females also rated odors as more intense in a study by Wysocki and Gilbert (1989), suggesting a higher sensitivity to odors.

The sex difference is generally more pronounced among the elderly because in males olfactory abilities start to decline at a younger age and/or decline more rapidly than in females (Doty et al., 1984; Wysocki and Gilbert, 1989; Ship et al., 1996). According to Ship et al. (1996), odor identification scores began to decline significantly at the age of 55 and 75 years among males and females, respectively. Interestingly, this pronounced sex difference in old age was not found in the healthy elderly screened for cognitive and olfactory dysfunction (Larsson et al., 2004). Thus, males may be more likely than females to have pathologies deteriorating olfaction in old age.

Although females' superior olfactory abilities appear clear, the causes for that are unknown. Several potential reasons have been suggested (reviewed by Brand and Millot, 2001): differences in anatomical, physiological, and hormonal factors, in cognitive abilities (especially in verbal processing), and in exposures to odors. From an evolutionary point of view, the females' olfactory advantage may have originally arisen from the need to protect offspring (Brand and Millot, 2001). This is supported by the finding that repeated exposures to odors sensitized only females of the reproductive age (Dalton et al., 2002).

Smoking

Frye et al. (1990) observed that smoking significantly deteriorates the ability to smell in a dose-related and reversible way. The higher the cumulative dose measured as pack-years (=the duration of smoking in years multiplied by the number of packs of tobacco smoked per day), the lower the odor identification score for both current and former smokers. Also, the longer the time since cessation of smoking, the higher the odor identification score. However, several studies, even some large population-based studies, have failed to reveal a significant relationship between smoking and olfactory abilities (e.g. Brämerson et al., 2004; Landis et al., 2004).

Diseases

Upper respiratory track infection (URTI) is probably the most common syndromic cause of olfactory dysfunction (Temmel et al., 2002). However, it is not clear

whether bacteria, viruses, or an immune responses directed against the OE is the causal agent of URTI (Landis et al., 2005). Sinonasal diseases and head trauma are other common causes of olfactory loss, together with idiopathic causes, i.e. causes unassociated with any disease (Temmel et al., 2002). Olfactory loss caused by head trauma is relatively permanent. In a study by Reden et al. (2006), only 10% of patients with posttraumatic olfactory loss experienced improvement in olfactory abilities over one year.

Olfactory impairment is a common symptom in such neurodegenerative diseases as Parkinson's disease (Doty, 2007b), Alzheimer's disease (Landis et al., 2005), and Huntington's disease (Moberg and Doty, 1997). Olfactory dysfunction is also associated with schizophrenia (Moberg, 1999), Down's syndrome, multiple sclerosis (Doty, 1997), and epilepsy (Landis et al., 2005). Olfactory disturbances (e.g. olfactory hallucinations) can occur with migraine during attacks or as an aura preceding attacks (Hirsch, 1992). Syndromic congenital anosmia occurs most frequently due to Kallmann's syndrome (Landis et al., 2005).

Exposure and olfactory plasticity

Olfactory plasticity refers to exposure-induced changes in olfactory abilities. Wysocki et al. (1989) found that systematic exposure to androstenone sensitized half of the subjects (who were initially insensitive to androstenone) to it. The authors proposed that changes in peripheral detection or central processing were responsible for the sensitization, but considered a shift in the cognitive processes to be an unprobable explanation. In an experiment by Mainland et al. (2002), exposing only one nostril to androstenone also sensitized the unexposed nostril. This provided evidence for the involvement of central components of the olfactory system in plasticity. In contrast, Wang et al. (2003) suggested that peripheral mechanisms explained the plasticity by showing that exposure to androstenone increased the olfactory evoked potentials and concomitantly decreased detection thresholds. Although the mechanisms are unclear, olfactory plasticity is likely not restricted to the perception of androstenone. Exposure-induced sensitization has also been demonstrated for benzaldehyde and citralva (Dalton et al., 2002) and androstadienone (Boulkroune et al., 2007).

Experience and learning

Responses to odors are modified by cognitive inputs. Culture-specific experiences influence intensity, pleasantness, familiarity, and edibility ratings for odors (Ayabe-Kanamura et al., 1998). Perceived intensity of odors appears to depend not only on the concentration of odorants but also on experience-dependent factors (Distel et al., 1999). An individual’s knowledge about the odor source influences pleasantness (Bensafi et al., 2007) and intensity ratings (Distel and Hudson, 2001) of odors. Even

false information (positive/negative label) has an effect on the pleasantness rating of an odor (Herz and von Clef, 2001). Emotional odor-associative learning can contribute to hedonic responses to odors; the emotional context in which an odor is encountered the first time becomes associated with that odor and the odor can later elicit the same emotion (Herz, 2005). Learning is a solution to cope with the vast diversity and unpredictability of the chemical world (Hudson, 1999). Thus, learning influences olfactory perception (reviewed by Stockhorst and Pietrowsky, 2004). The purported superior ability of chefs, sommeliers, and perfumers (as well as trained animals such as sniff dogs) to discriminate odors is obviously due to learning rather than inherent differences in the olfactory system (Firestein, 2001). Indeed, learning appears to play a central role especially in odor discrimination (Wilson and Stevenson, 2003). Olfaction is suggested to be more dependent on experience than other senses (Stevenson and Boakes, 2003). Hudson (1999) thus concluded that an adequate understanding of the olfactory function is not possible without taking experience-dependent factors into account.

2.2 Genetics of olfaction

The genes most directly linked to olfaction are the genes encoding ORs. In addition, some genetic elements may have their effects on the central processing of the olfactory signals (Wysocki and Beauchamp, 1984; Gross-Isseroff et al., 1992; Menashe et al., 2007; Pinto et al., 2008). However, as the OR genes are the best-known genes influencing olfaction, this review focuses on these, after introducing some basic concepts of human genetics.

2.2.1 The human genome and genetic variation

Genetic information

Genetic information is coded with four nucleotides of deoxyribonucleic acid (DNA). These nucleotides are named after their nitrogenous base parts: adenine (A), guanine (G), cytosine (C), and thymine (T). DNA normally occurs as a lengthwise combination of two complementary molecules (double-stranded DNA), the length of which is described using base pair (bp) and kilo base pair (kb) units. In the nucleus of a cell, very long helical DNA molecules form structures called chromosomes.

Normal human cells (except for the gametes, viz., ovum and sperm) have 46 chromosomes: a pair of each of 22 autosomal chromosomes (autosomes) and a pair of sex chromosomes (X, Y). Females have two X chromosomes, while males have one X and one Y chromosome. These cells are diploid, whereas the gametes are haploid; they have only a single copy of each autosome and one sex chromosome.

An ovum has always an X chromosome, while a sperm has either an X or a Y chromosome. One chromosome of each chromosome pair (of homologous chromosomes) is inherited from the mother (maternal) and the other comes from the father (paternal). Homologous chromosomes are similar in length and sequence, thus, humans have two copies of every gene (except for those on the sex chromosomes). The homologous chromosomes (sister chromatids) are attached to each other at the centromere, which divides the chromosome into two arms. The shorter of the arms is labeled p and the longer q (after the French words 'petit' [small] and 'queue' [tail], respectively). The tips of the arms are called telomeres. The arms are divided into bands, which are numbered from the centromere. The rough location of a gene can thus be specified by the chromosome number, the arm, and the band (e.g. 4q32.3) (Strachan and Read, 2004).

Genome refers to the totality of DNA characteristic of a species. The human genome comprises about $3*10^9$ bp of DNA per haploid nucleus. However, only a small fraction of DNA encodes for functional RNA and proteins. The vast majority is noncoding with an unknown function. Within a gene, the DNA segments that encode for a protein are called exons. The possible noncoding segments between the exons are called introns (intervening sequences). Introns and noncoding segments in intergenic regions have been called junk DNA, but they may nevertheless have important functions, e.g. in gene regulation (Sham, 1998).

Gene denotes the segment of the DNA that encodes functional ribonucleic acid (RNA). The RNA is produced according to the information in the nucleotide sequence of the DNA in a cellular process called transcription. The genetic code is read from the DNA as the sequential triplets of bases (codons) in defined locations (reading frame). Each of the $4^3=64$ codons corresponds to an amino acid or a termination signal (stop codon). The genetic code is said to be degenerate to denote that more than one codon (up to six) can code for the same amino acid. The introns are removed from the primary transcript in a process called RNA splicing. Several classes of RNA have specific functions in the cells. The messenger RNA (mRNA) transmits the information of the DNA to the amino acid sequence of a polypeptide in a process called translation. Thus, the nucleotide sequence of a gene can determine the structure and function of a protein. Genes vary enormously in length (Sham, 1998; Strachan and Read, 2004).

The term gene was first introduced long before the discovery of DNA. Increased understanding of the structure and function of the DNA together with an ambiguous use of the term have made it difficult to define the term gene comprehensively (Sham, 1998). Defective copies of functional genes are called pseudogenes (Strachan and Read, 2004).

The position of a specific gene within the genome is constant. A specific position (of a gene or any other segment of DNA) in the genome is defined as a locus. Alternative DNA sequences (e.g. alternative forms of a gene or a genetic marker) at a locus are called alleles (Sham, 1998). Different alleles of a gene may have different effects on phenotype. When the same allele is expressed differently in different environments, genetic and environmental effects are said to interact (Posthuma et al., 2003). One gene may affect more than one trait, a phenomenon known as pleiotropy. In turn, the interaction of two genes at separate loci in which one gene suppresses the influence of the other on a phenotype is known as epistasis.

Genetic variation

Genomic DNA of individuals differs, on average, in about one out of 1000 bases (average nucleotide diversity). Genetic variation among a population originates from mutations, heritable changes in DNA. Several types of mutations introduce diversity into the population. Substitutions involve replacement of bases. In deletions bases are removed and in insertions added into a DNA sequence. Deletions and insertions can result in a shift in the translational reading frame (frameshift mutation), usually eliminating the production of a functional protein. Often only one nucleotide is changed, most frequently substituted by a different nucleotide, introducing a single nucleotide polymorphism (SNP) (Strachan and Read, 2004).

Generation of mutations is mostly endogenous and essentially random, thus coding and noncoding DNA are about equally susceptible to mutations. However, consequences of mutations are mainly restricted to coding DNA. Synonymous (silent) mutations do not change the amino acid sequence of the gene product. By contrast, nonsynonymous mutations change the sequence of the gene product; the altered codon specifies the altered amino acid (missense mutation) or stop codon (nonsense mutation). The effect of novel nonsynonymous mutations can be neutral or beneficial, but most often deleterious. Their population frequency is therefore greatly reduced because of natural selection, resulting in a much lower overall mutation rate in coding DNA than noncoding DNA (evolutionary conservation of coding DNA). The process of removing deleterious mutations from the population is called negative (or purifying) selection. In the rare case that the mutation is beneficial, it is subjected to positive (or advantageous) selection (Strachan and Read, 2004).

Genetic variation between individuals arises from rearrangement of genetic material in meiosis (cell division that produces gametes). Recombination (crossing-over) refers to an exchange of DNA segments between the maternal and paternal sister chromatids (i.e. between homologous chromosomes) during meiosis. This reshuffling produces sister chromatids that consist of alternating DNA segments of maternal and paternal origin. Pairs (sister chromatids) of the newly reconstituted 23

chromosomes are then independently assorted into two daughter gametes, allowing 2^{23} (~$8.4*10^6$) different combinations of chromosomes. The recombination and independent assortment of homologous chromosomes together result in the potential to form an almost infinite number of genetically different gametes (Strachan and Read, 2004).

The recombination fraction (θ) denotes the proportion of meioses in which the two loci are separated by recombination. The nearer the two loci reside to each other in the same chromosome, the smaller the recombination fraction. It varies between 0 and 0.5 and is always 0.5 for loci in different chromosomes. Haplotype refers to a specific combination of alleles at loci so near each other that they are likely to be inherited as a single unit and not separated in recombination (Strachan and Read, 2004).

2.2.2 Olfactory receptor genes

Discovery

The OR gene family was discovered as a result of the pioneering work by Nobelists Linda Buck and Richard Axel. In their fundamental paper, Buck and Axel (1991) identified the first potential OR genes in rats. They demonstrated that ORs are encoded by a multigenic family, are expressed in the OE, and belong to the GPCR superfamily. They also characterized conserved and variable regions in the sequences of several OR genes and found that variable regions could be conserved within a smaller group of genes, which was consequently classified into the same subfamily.

Structure and classification

OR genes of vertebrates contain no introns (Buck and Axel, 1991; Breer, 2003). Thus, an OR gene is expressed as a single transcript and no alternative splicing can occur. Invertebrate OR genes, by contrast, may contain introns (Breer, 2003). Nucleotide sequences of OR genes include conserved nucleotide sequence motifs, and thus, ORs contain conserved amino acid sequence motifs (see Section 2.1.2).

Some human OR genes occur entirely in an intact form, resulting in functional OR proteins, and some entirely in a pseudogenic, nonfunctional form that either is not expressed at all or results in a nonfunctional OR. Moreover, functional OR genes may display several alleles that all result in functional ORs, which may, however, have highly differing affinities to odorants.

It is noteworthy that the classification of OR genes as functional genes or pseudogenes based on the gene sequence is only tentative. Final assignment of the OR gene status should be made using expression data (Zozulya et al., 2001). A

putatively functional OR gene containing an intact coding region may be nonfunctional due to other reasons, e.g. because of mutations in the promoter region that prevent the expression of the gene (Gilad and Lancet, 2003). In addition, an intact OR gene can result in a nonfunctional OR protein due to missense mutations in functionally important regions (Menashe et al., 2006). In this text, both putatively functional and pseudogenic forms of OR genes are referred to collectively as OR genes unless otherwise specified.

Interestingly, some OR genes display both functional (wild type), functional mutated and nonfunctional alleles, and thus, are called segregating pseudogenes (SPGs; Menashe et al., 2003). Occurrence of SPGs may be indicative of an ongoing process of deterioration of OR genes, probably due to relaxed evolutionary constraints in humans (Gilad et al., 2003a). SPGs confer substantial potential for variation in odor perception, as SPGs constitute both natural knockout alleles with no contribution to detection of any odorant and potential alleles for ORs with a high affinity (wild type alleles) or altered affinity (mutated alleles) to specific odorants.

Gilad and Lancet (2003) identified 12 SPGs in one human sample. They found higher frequencies of intact alleles in an African population (Pygmies) than in Caucasians. Menashe et al. (2003) identified 18 SPGs and observed great diversity in the OR gene repertoire among individuals. Also, African American subjects were found to have more functional OR genes than non-African participants. The total number of human SPGs has been extrapolated to be at least 60 (Menashe et al., 2003).

Mammalian OR genes can be classified into subfamilies based on their nucleotide sequences (Breer, 2003). Malnic et al. (2004) classified human OR genes into 172 subfamilies, each subfamily including 1-9 genes. These subfamilies were found to be located in 51 genomic loci. One locus normally includes all members of one subfamily and contains up to 35 subfamilies.

Phylogenetically, OR genes are classified as Class I, fish-like, or as Class II, mammalian-like (or tetrapod-specific) OR genes. Class I genes were initially found in fish, but they have also been identified in other species, including humans. Of the human OR genes 10% (Glusman et al., 2001) to 13% (Niimura and Nei, 2003) belong to Class I and the remaining ~90% to Class II.

Number of OR genes and pseudogenes

Individual OR genes have been identified in tens of species (Glusman et al., 2001). However, only recently, as the whole genome sequence information has become available for the human and several other species, has it been possible to determine the total number of OR genes for respective species.

Estimates for total number of OR genes can be achieved using data mining, i.e. systematic searches of genome sequence databases. Putative OR genes are identified by their conserved sequences. Due to differences in identification methods and completeness of the genome sequence data used, some variability exists in estimates even for the same species across studies. The first estimates for a species were often made using an incomplete version of the genome sequence; thus, the more recent works present more reliable numbers. However, the definite number of OR genes can be achieved only after the functionality of potential genes has been demonstrated. Studies reporting the number of OR genes in the human genome and that of some other species are listed in **Table 1**.

OR genes constitute the largest gene superfamily in vertebrates (Glusman et al., 2001). The human genome includes ~850 OR genes, ~390 of which are potentially functional genes, the remainder being pseudogenes. However, as discussed above, the proposed numbers of functional and nonfunctional OR genes (and ORs) are only tentative until expression of potential OR genes (and functionality of ORs) has been demonstrated. Using a probabilistic classifier, Menashe et al. (2006) estimated that ~135 of the putatively functional human OR genes yield nonfunctional ORs caused by missense mutations. Thus, the human genome may have only ~250 OR genes that produce functional ORs.

Of the putatively functional genes, Niimura and Nei (2003) classified 57 (15%) into Class I and 331 (85%) into Class II. Of the pseudogenes, they classified 45 (11%) into Class I and 369 (89%) into Class II. According to Glusman et al. (2001), the proportion of pseudogenes among Class I OR genes (52%) is considerably lower than that of Class II OR genes (77%). More recently, Niimura and Nei (2003) also found that the proportion of pseudogenes to be lower among Class I (44%) than Class II (53%) OR genes, although the proportions were lower in both classes than estimated by Glusman et al. (2001).

Table 1. ***Number of putative OR genes in humans according to sequence database mining studies and comparisons with selected other species.***

Species	Number of OR genes			Proportion of pseudo-genes (%)	Reference
	Functional	Pseudo	Total		
Human	388	479	867	55	Gloriam et al., 2007
	387	415	802	52	Niimura and Nei, 2007
	388	414	802	52	Niimura and Nei, 2003
	339	297	636	47	Malnic et al., 2004
	322	584	906	64	Glusman et al., 2001
	347	- [b]	- [b]	- [b]	Zozulya et al., 2001
Chimpanzee	353	546	899	61	Gilad et al., 2005
Macaque	309 + 17[a]	280	606	46	Niimura and Nei, 2007
Dog	811 + 11[a]	278	1100	25	Niimura and Nei, 2007
Cow	970 + 182[a]	977	2129	46	Niimura and Nei, 2007
Rat	1234	552	1786	31	Gloriam et al., 2007
	1207 + 52[a]	508	1767	29	Niimura and Nei, 2007
Mouse	1081	325	1406	23	Gloriam et al., 2007
	1035 + 28[a]	328	1391	24	Niimura and Nei, 2007
	913	296	1209	24	Godfrey et al., 2004
	1037	354	1391	25	Niimura and Nei, 2005a
	1036	260	1296	20	Zhang and Firestein, 2002
Opossum	1188 + 10[a]	294	1492	20	Niimura and Nei, 2007
Platypus	265 + 83[a]	370	718	52	Niimura and Nei, 2007
Frog	410	478	888	54	Niimura and Nei, 2005b
Zebrafish	143	10	153	7	Alioto and Ngai, 2005
	98	35	133	26	Niimura and Nei, 2005b
Pufferfish (Fugu)	44	4	48	8	Alioto and Ngai, 2005
	40	54	94	57	Niimura and Nei, 2005b
Tetraodon	42	11	53	21	Alioto and Ngai, 2005

[a] Number of probably intact genes that appear truncated due to gaps in sequence data.
[b] Only putatively functional OR genes identified.

In humans, a relatively large fraction, over half, of OR genes are of pseudogenic form, while the respective fraction is only ~25% in the mouse (Niimura and Nei, 2007; Gloriam et al., 2007) and dog (Niimura and Nei, 2007) and ~30% in the rat (Gloriam et al., 2007; Niimura and Nei, 2007) (**Table 1**). Gilad et al. (2003a) compared gene silencing of 50 randomly selected OR gene sequences in primates and found that the proportion of pseudogenes was 54% in humans and only 28%, 32%, 32%, and 36% in the gorilla, chimpanzee, orangutan, and rhesus macaque, respectively. Using similar methods, Gilad et al. (2004) further showed that the fraction of OR pseudogenes was ~30% in Old World monkeys and only ~20% in New World monkeys. The relatively high proportion of pseudogenes in humans suggests a reduced importance of olfaction for the human than for the other aforementioned species.

Copy number variation

Copy number variation (CNV) is a form of structural variation of DNA due to insertion, deletion, duplication, or more complex rearrangement of DNA segments >1 kb in size (Redon et al., 2006). As its name implies, CNV among individuals is typically due to variation in the number of copies of specific genes (Conrad and Hurles, 2007). Redon et al. (2006) identified 1447 regions in which CNV occurs in the human genome (copy number variable regions), and Nozawa et al. (2007) found that 3144 of ~22 000 human genes (~14%) show CNV (copy number polymorphic genes). CNV may have important manifestations in complex diseases (McCarroll and Altshuler, 2007).

CNV is particularly common among OR genes. According to Nozawa et al. (2007), ~30% of the human OR genes were polymorphic with respect to CNV. They found no significant differences in the CNV amounts between functional and nonfunctional OR genes. In contrast, individual variation in CNVs of OR genes and pseudogenes was substantial; the mean difference between two individuals was ~10 gene copies. Thus, in addition to variation in the number of different OR genes and their allelic variation, CNV also appears to play an important role in the genetics of olfaction. However, the relation of CNV to odor perception remains to be evaluated.

Genomic distribution

OR genes are located on 22 human chromosomes; only chromosomes 20 and Y are devoid of them (Glusman et al., 2001; Niimura and Nei, 2003). The distribution of OR genes is uneven; the six chromosomes that are most rich in OR genes (1, 6, 9, 11, 14, and 19) contain about three-fourths (73%) of them (Glusman et al., 2001). Chromosome 11 harbors nearly half (42%) of the OR genes (Glusman et al., 2001).

A characteristic feature of OR genes is their organization into clusters. Glusman et al. (2001) observed 24 clusters with six OR genes or more, and 78% of the OR genes in their data were included in these clusters. They also demonstrated that no clusters including more than five genes would be expected if the genes were distributed randomly. Niimura and Nei (2003) identified 30 OR gene clusters that included at least six OR genes, while only 29 OR genes were found to exist separately.

Glusman et al. (2001) showed that none of the clusters, except one, contained an unexpected number of OR pseudogenes and concluded that the OR gene disruption is a random process targeted at individual genes. Except for one large OR gene cluster on chromosome 11, the clusters do not include non-OR genes (Glusman et al., 2001).

All Class I OR genes (putatively functional genes and pseudogenes) are located on chromosome 11 in a large cluster at locus 11p15.4 (Glusman et al., 2001; Niimura and Nei, 2003). Chromosome 11 also contains some Class II genes (Glusman et al., 2001), but not in the same cluster as the Class I genes (Niimura and Nei, 2003). The OR genes belonging to the same phylogenetic clade are usually located close to each other on a chromosome, but can often be found in more than one cluster (Niimura and Nei, 2003).

Expression

Expression of an OR gene is a requirement for its functionality. The early work of Buck and Axel (1991) demonstrated that a set of rat OR genes was expressed in the OE, but not in other studied tissues. Recently, Zhang et al. (2007) designed a novel DNA microarray and used it to examine expression of nearly all (578) putative human OR genes. Of these, 437 (76%) were found to be expressed in the OE, promoting functionality of these genes. Most intact OR genes (80%) were expressed in the OE. Interestingly, also 67% of OR pseudogenes showed expression in the OE, however, at a lower level, on average, than the intact OR genes. In addition, 32 OR genes were expressed in a nonolfactory tissue (liver, lung, kidney, heart, or testis), but not in the OE. However, no support for additional functions of these ORs was found. Zhang et al. (2007) also observed inter-individual variation in the repertoire of the OR genes which were expressed and hypothesized that variation in regulation of OR gene expression contributes to variation in olfactory sensitivity among individuals.

Association with perception of odors

Ligand specificity of several ORs has been resolved using cell models (reviewed by Mombaerts, 2004). However, only two specific OR genes have been associated with psychophysically measured responses to specific odorants to date.

Keller et al. (2007) screened first responses of the 335 human ORs to androstenone using a cell-based assay and found that receptor OR7D4 showed the strongest response. They then screened responses of the most common allele of OR7D4 (denoted OR7D4 RT) to a set of 66 odors and observed that, in addition to androstenone, OR7D4 RT also responded to a structurally related odorant, androstadienone. In contrast, another common variant of the receptor, OR7D4 WM, was not activated by androstenone or androstadienone. Further, the authors compared responses to androstenone and androstadienone among subjects with different genotypes for the OR7D4. They found that the *RT/WM* group rated the odors of androstenone and androstadienone (but none of the other 64 odors) as less intense and less unpleasant than the *RT/RT* group (suprathreshold scaling). Consistent with these findings, the *RT/WM* group had higher detection thresholds for both odorants and was more likely to describe androstenone as smelling like "vanilla" than the *RT/RT* group.

Menashe et al. (2007) explored associations between genetic variation in 43 human OR SPGs (displaying both intact and pseudogenic alleles) and detection thresholds of four odorants (isoamyl acetate, isovaleric acid, L-carvone, and cineole). They observed a strong association between forms of the *OR11H7P* gene and sensitivity to isovaleric acid. Individuals heterozygous or homozygous for the intact allele (*OR11H7Pi*) were more likely to be hyperosmic to isovaleric acid than individuals homozygous for the disrupted allele. The responsiveness of the receptor encoded by the intact allele to isovaleric acid was confirmed using cell-based assay. The authors suggested that hyperosmia to isovaleric acid is a complex trait influenced by both receptor genes and other genetic factors with a more general effect on sensitivity to odors.

2.2.3 Evolution of the olfactory receptor gene superfamily

The OR gene superfamily has undergone substantial changes during evolution. The number and diversity of OR genes as well as the fraction of pseudogenes appear to reflect requirements imposed by the environment of a given species. The large size of the OR gene superfamily and its dynamic nature make exploring evolution of olfaction feasible (Gilad et al., 2003b).

Mutations that disrupt intact OR genes tend to accumulate faster in humans than in nonhuman primates. Gilad et al. (2003a) found that the rate of gene disruption (gene

silencing rate) in humans was roughly fourfold that in nonhuman primates (chimpanzee, gorilla, orangutan, and rhesus macaque). They proposed that a decreased need for olfaction had resulted in relaxed evolutionary constraints and a higher OR gene silencing rate in humans.

The evolution of vision appears to have diminished the role of the sense of smell. Gilad et al. (2004) explored the proportion of OR pseudogenes in 19 primate species and observed that the species with full trichromatic vision (humans, apes, Old World monkeys, and howler monkey) also have a larger fraction of OR pseudogenes than species with dichromatic vision (New World monkeys, excluding howler monkey, and lemur). They concluded that disruption of a substantial fraction of functional OR genes coincided with the acquisition of full trichromatic vision, but identification of the lost genes could not be accomplished, as the whole genome sequence for most species was not available at that point.

Although a larger fraction of OR genes have lost their functionality during evolution in humans than in non-human primates (Gilad et al., 2003a, 2004), some human-specific variants of OR genes may also have evolved. Gilad et al. (2003b) suggested that a subset of OR genes has been under positive selection in the human lineage. In other words, these genes have gained mutations that are beneficial for humans, and thus, these new gene variants have been favored, not purified, in human evolution. Gilad et al. (2003b) found that stronger positive selection was acting on human relative to chimpanzee OR genes. They speculated that this may be due to development of novel olfactory needs unique to humans as a result of cooking.

Most human OR gene subfamilies (87%) have a counterpart in the mouse. However, most common subfamilies have more members in the mouse. Thus, mice may have a better ability to discriminate closely related odorants than humans. In addition, mice have more species-specific subfamilies than humans. Unique subfamilies have been suggested to encode ORs for detection of odorants of selective importance, such as pheromones (Godfrey et al., 2004). Niimura and Nei (2005a) determined that, compared with humans, mice have up to 5.6 times more genes in related OR gene clusters, but the same clusters were still present in both species.

The OR gene superfamily has expanded far more extensively in the mouse than in the human lineage since diverging from their most recent common ancestor (MRCA). Niimura and Nei (2005b) estimated that the MRCA had 754 functional OR genes. Roughly half of these have been eliminated from the genome or have become pseudogenes in the human lineage. In contrast, most OR genes of MRCA remain functional in the mouse lineage. In addition, the mouse gained ~350 new OR genes after the human-mouse divergence, while humans have acquired only ~60 (Niimura and Nei, 2005b). Thus, the difference in the number of OR genes between

humans and mice emerges mainly from gene expansion in the mouse lineage and gene loss in the human lineage.

2.2.4 Inheritance of olfactory-related traits

Comparison of twins

Evidence of genetic influence on olfactory-related traits has been derived mainly from studies comparing (within-pair) intraclass correlations between monozygous (MZ) and dizygous (DZ) twins. A higher correlation among MZ than DZ twins indicates a genetic influence. In twin studies, detection thresholds of odors have been most frequently explored, whereas other olfactory traits have been covered only in a few studies (**Table 2**).

Using twin research, the most convincing evidence of genetic effects to date has been demonstrated for the odor detection threshold of androstenone (Wysocki and Beachamp, 1984; Gross-Isseroff et al., 1992; Pause et al., 1998). The results were subsequently confirmed by the identification of one underlying OR gene (Keller et al., 2007). Some evidence of a genetic influence has also been found for the odor identification ability (Segal et al., 1995; Finkel et al., 2001). Support for this was provided in a recent report showing suggestive evidence of a susceptibility locus for hyposmia, measured using the odor identification test, on chromosome 4q (Pinto et al., 2008).

Heritability estimates

Heritability refers to the relative contribution of genetic effects to the variation of a trait (see Section 2.3 for details). Finkel et al. (2001) determined heritability estimates for several olfactory-related traits. Odor identification showed a significant, moderate heritability (29%), whereas for other traits the estimates were lower and not significant (**Table 2**).

Mode of inheritance

The mode of inheritance of olfactory-related traits has been studied relatively little. However, considering that most of these traits are quantitative by nature, after the discovery of a multitude of odorant receptor genes and combinatorial olfactory coding, it has become increasingly obvious that the traits follow polygenic inheritance. Perhaps only in the case of a clear-cut specific or congenital general anosmia should simple inheritance patterns be considered.

Table 2. ***Results from twin studies of responses to odors.***

Studied trait	Evidence of genetic component[a] (+heritability estimate if determined)	Number of twin pairs		Reference
		MZ	DZ	
Detection threshold				
-androstenone	suggestive	15	15	Pause et al., 1998
-isoamyl acetate	suggestive			
-citral	no			
-phenyl ethyl alcohol	no	46	37	Segal et al., 1995
-androstenone	yes	17	15	Gross-Isseroff et al., 1992
-isoamyl acetate	yes			
-citral	no			
-eugenol	no			
-androstenone	yes	17	21	Wysocki and Beauchamp, 1984
-pyridine	no			
-acetic acid	no	51	46	Hubert et al., 1980
-isobutyric acid	no			
-cyclohexanone	no			
Detection (suprathreshold)				
-yes/no for odors in NatGeo[b]	no (14%)	86	141	Finkel et al., 2001
Identification				
-NatGeo[b]	yes (29%)	86	141	Finkel et al., 2001
-UPSIT[c]	yes in males, no in females	46	37	Segal et al., 1995
Intensity				
-mean of odors in NatGeo[b]	suggestive (25%)	86	141	Finkel et al., 2001
Pleasantness				
-mean of odors in NatGeo[b]	no (17%)	86	141	Finkel et al., 2001

[a] Significantly ($p<0.05$) higher intraclass correlation in MZ twin pairs than in DZ twin pairs was regarded as evidence of a genetic component.
[b] *National Geographic* Smell Test, (six odors: isoamyl acetate, eugenol, rose, mercaptan mixture, androstenone, and Galaxolide; Wysocki and Gilbert, 1989), Swedish translation.
[c] University of Pennsylvania Smell Identification Test (40 odors; Doty et al., 1984).

A simple recessive autosomal inheritance was suggested for specific anosmia to pentadecalactone (musk), based on the results of a pedigree analysis of 109 two-generation Caucasian families (Whissell-Buechy and Amoore, 1973). However, the authors acknowledged that polygenic inheritance could not be ruled out. Furthermore, recent reports on associations between odorant receptor genes and detection thresholds of odorants describe it as likely that more than one gene modifies the traits (Keller et al., 2007; Menashe et al., 2007).

Congenital general anosmia in otherwise healthy individuals (isolated congenital anosmia) is very rare, but tends to cluster in families, which implies a genetic origin. Autosomal dominant inheritance with incomplete penetrance was proposed for the trait by Ghadami et al. (2004). In addition, they found a significant genetic linkage (LOD score 5.1) for the trait on chromosome 18, but failed to identify the underlying gene among the eight candidate genes studied. The same mode of inheritance was also proposed in a subsequent study, but no causative mutations were identified in the three main olfactory transduction genes investigated (Feldmesser et al., 2007). The possibility of an oligogenic background for the trait was presented in both studies.

2.3 Strategies to uncover the genetic background of olfaction

Research on the genetic background of a quantitative trait usually begins with exploring the variation of the trait. Substantial phenotypic variation (distinguished from random variation due to measument error) in the trait makes it interesting to study the relative contributions of genetic and environmental factors to the variation. A genetic component of variation can be determined using heritability analysis. Its results indicate how effectively genes modify the trait. At least moderate heritability of a trait warrants a search for underlying genetic loci. This can be achieved using genetic linkage analysis. This analysis can lead to the discovery of involved genetic loci and candidate gene(s) or other genetic elements suspected of controlling the trait. If genetic elements linked to the trait are found, variants (alleles) of these are then worth exploring. When alleles are known, their association with the variation in the trait can be investigated using association analysis.

2.3.1 Heritability

Heritability refers to the proportion of phenotypic variance in a trait that is attributable to genetic factors (genetic variation). Total phenotypic variance (V_p) in a trait is the sum of variance due to genetic variation (V_g) and environmental variation (V_e), and covariance between these two (cov_{ge}):

$$V_p = V_g + V_e + 2cov_{ge}$$

The covariance term ($2cov_{ge}$) is often omitted form quantitative genetic models (Posthuma et al., 2003). Variation due to measurement error should be added to this theoretical equation when the variables are measured.

Heritability (H^2) can be expressed as the ratio of genetic variance over total variance:

$H^2 = V_g / V_p$

More precisely, this denotes broad-sense heritability. The phenotypic variance attributable to genetic variation can further be divided into components due to additive and dominant effects, and due to epistasis. Dominant genetic variance arises from deviations from purely additive genetic effects of alleles. Epistasis arises from interaction of alleles at different loci.

Narrow-sense heritability (h^2) is defined as the proportion of phenotypic variance attributable to additive effects of genetic variation (additive genetic variance, V_a):

$h^2 = V_a / V_p$

Narrow-sense heritability is widely used for a parameter that describes magnitude of genetic influence on a trait. In practice, if the heritability estimate for a trait is, for instance, 60%, this means that 60% of the variation of the trait is explained by genetic factors. It is important to note that heritability is a population-level parameter. Since the estimation of heritability is based on a measure of variation across the population, heritability estimates are applicable only to this population overall, not necessarily to each individual separately. It should also be noted that additive genetic variance is not necessarily the only source of resemblance between relatives (Lynch and Walsh, 1998).

Heritability is a measure of inheritance of a trait, and its estimation thus requires data from relatives. Heritability estimates can be calculated from family data. However, shared environmental effects tend to inflate the heritability estimate, as environmental exposure is often greater among relatives than among unrelated individuals. Shared environmental effects may therefore mimic genetic similarity among relatives, leading to overestimates of heritability. This problem is circumvented when heritability estimates are calculated using quantitative genetic modeling of data from twins.

2.3.2 Quantitative genetic modeling

Comparison of MZ and DZ twins is widely used for estimating relative contributions of genetic and environmental effects to total phenotypic variation of a trait

(Boomsma et al., 2002). Twin comparisons rely on the assumption that MZ twins share all and DZ twins, on average, half of their segregating genes, and also on the assumption that the environment shared by a twin pair influences twins similarly regardless of their zygosity (equal environment assumption). Thus, higher within-pair intraclass correlation (resemblance) among MZ twins than among DZ twins indicates genetic contribution (heritability) to the trait under study. Since MZ twins are assumed to share their genes and a part of their environment (shared, common environment), any resemblance between the twins is attributed to these, while any difference between them is attributed to nonshared (specific, individual) environmental factors, which, by definition, also include measurement error. Resemblance between DZ twins is also due to their shared genes and the environment, but because DZ twins share, on average, only half of their genes, any resemblance between them due to genetic effects is smaller than for MZ twins (Posthuma et al., 2003).

Contrary to the assumption, MZ twins are not always genetically identical. MZ twins can differ at the level of DNA sequence and at the level of chromosomes (the number or morphology of chromosomes may vary) (Gringras and Chen, 2001). Bruder et al. (2008) observed CNVs within MZ pairs. Furthermore, MZ twins may have epigenetic differences, i.e. patterns of gene expression may vary within MZ pairs (Petronis, 2006).

Data from MZ and DZ twins allow one to decompose the phenotypic variance into components of additive genetic variance, nonshared environmental variance, and either dominant genetic variance or shared environmental variance. The latter two cannot be estimated simultaneously if only data from twins reared together are included.

Rough estimates of relative contributions of various sources of variation to phenotypic variation can be calculated from the following equations (where r_{MZ} = intraclass correlation within MZ twin pairs, r_{DZ} = intraclass correlation within DZ twin pairs):

-proportion of additive genetic effects = $a^2 = 2(r_{MZ} - r_{DZ})$

-proportion of dominant genetic effects = $d^2 = 2r_{MZ} - 4r_{DZ}$

-proportion of shared environmental effects = $c^2 = 2\ r_{DZ} - r_{MZ}$

-proportion of nonshared environmental effects = $e^2 = 1 - r_{MZ}$

The correlational method merely provides parameters. Decomposition of phenotypic variance into genetic and environmental components and estimation of their relative contributions can also be performed using computer-aided structural equation

modeling of twin data. This method has an advantage that it allows determination of confidence intervals for parameters and provides information about goodness-of-fit of the models, i.e. how well the specified model describes the data (Posthuma et al., 2003). To obtain reliable results, the assumptions of the modeling, viz., equal means and variances between MZ and DZ twins and between twins within a twin pair, should be valid. However, the validity of the assumptions can be tested statistically. Basic twin models also assume that there is no gene × environment interaction, epistasis, or assortative mating (correlation between phenotypic values of spouses).

A univariate model can be used to estimate (narrow-sense) heritability of a trait. Moreover, a bivariate model can be used to determine the genetic correlation between two traits, i.e. to estimate how much the two traits are influenced by the same genes. The twin intraclass correlations (r_{MZ} and r_{DZ}) are usually calculated first to give a hint as to whether a shared environmental component (C) or a dominant genetic component (D) should be modeled together with an additive genetic component (A) and a nonshared environmental component (E). If the ratio r_{MZ}/r_{DZ} exceeds two, it implies that the component D would be more important than the component C. If the ratio is less that two, the reverse is true. Starting with either an ACE or ADE model, the significance of A, C, and D can then be tested. Nonsignificant components can be omitted from the model when a more parsimonious model (AE/CE/E) is sought. However, a DE model is not biologically plausible and the E component cannot be omitted because it includes the measurement error. More complex models are reviewed by Posthuma et al. (2003) and Neale and Maes (2004).

2.3.3 Search for underlying genetic loci

The next step in exploring the genetic background of a trait, after it has been found to be at least to some extent heritable, is often to search for the underlying genetic loci and genes. If the influential gene(s) cannot be discovered based on specific information (such as ligand specificity screening of ORs using cell models) a hypothesis-free approach of a genome-wide screen can be used. A genome-wide screen for mapping effective loci can be performed using the genetic linkage analysis.

Linkage analysis, as its name implies, is based on the phenomenon of genetic linkage. Alleles at loci on separate chromosomes (nonsyntenic loci) are transmitted to the next generation independently. In contrast, alleles at loci on the same chromosome (syntenic loci) can display a linkage. This means that syntenic loci are more likely to be transmitted together to an offspring than nonsyntenic loci. If the recombinant

fraction (θ) between two syntenic loci is less than 0.5, the loci are said to be in linkage. The smaller θ, the tighter is the linkage between the two loci (Sham, 1998).

Marker maps in linkage analysis employ centimorgan (cM) as a unit for genetic distance. It measures relative genetic distance between loci. If two loci are 1 cM apart, they have a 1% probability of recombination during meiosis (Strachan and Read, 2004).

In linkage analysis, potential linkages between markers (typically short tandem repeats, also called microsatellite markers) and loci underlying the genetic effect on the studied trait are sought. A standard set of about 400 markers (corresponding to a marker map density of 10 cM) is normally used. If a linkage to a marker is found, it provides evidence that a locus associated with the studied trait resides near this marker. When evaluating the statistical significance of a potential linkage, the null hypothesis is that no linkage exists between the marker locus and the potential influential locus, and the alternative hypothesis is that linkage exists between the two. The statistical significance of a potential linkage is frequently evaluated using the logarithm of odds (LOD) score:

LOD score = $\log(L_\theta/L_{\theta=0.5})$,

where L_θ is the value of the likelihood function when θ maximizes the function, and $L_{\theta=0.5}$ is the value of the function when $\theta = 0.5$. When a standard set of 400 markers is employed, a LOD score of three is a commonly applied as a rough criterion for genome-wide significance of linkage. It derives from the fact that if the criterion for genome-wide significance is selected to be $\alpha=0.05$, then the corresponding pointwise significance should be $p=0.0001$ according to the correction for 400-fold multiple testing (Bonferroni correction). Lander and Kruglyak (1995) proposed that a LOD score of 3.3 be the threshold for a significant linkage and a LOD score of 1.9 for suggestive linkage. It is also common practice to set the criterion for significance empirically by simulating a large number (≥100) of linkage analyses using the given data.

3 AIMS OF THE STUDY

This study aimed to determine the genetic and environmental contributions to several olfactory-related traits and to localize the underlying genetic loci.

The specific aims addressed in the family (I) and twin (II-IV) samples were:

- To estimate heritability of odor identification ability (I, IV) and mean perceived intensity of odors (IV)
- To estimate heritability of perceived intensity and pleasantness of individual odors (I-III)
- To localize potential genetic regions modifying the odor identification and responses to individual odors (I)
- To estimate heritability of self-rated olfactory function and odor annoyance and to explore factors associated with the odor annoyance (IV)
- To explore associations of nongenetic factors (e.g. sex, age, and smoking) with odor identification (I, IV), perceived intensity and pleasantness of individual odors (I-III), and self-rated olfactory function and odor annoyance (IV)

4 MATERIALS AND METHODS

Materials and methods of the study are summarized here and described in more detail in original publications I-IV.

4.1 Subjects

4.1.1 Finnish families (I)

The subjects of the Finnish family study (I) were a subsample of the Finnish migraine family study (Wessman et al., 2002). This subsample consisted of 146 adult Finns, of whom 100 were females and 46 males. These individuals were derived from 26 families with familial migraine (with or without aura). Most participants (84%) suffered from clinically diagnosed migraine, while the rest (16%) were their healthy relatives. The subjests were aged from 18 to 78 years (mean age 49 years). Genome-wide marker data from these subjects (350 microsatellite markers, acquired as described in Wessman et al., 2002) were available.

4.1.2 Finnish twins (II-IV)

The Finnish twins were derived from the FinnTwin 12 (FT12) cohort study (Kaprio et al., 2002; Kaprio, 2006). Data from 219 twin individuals (138 females and 81 males) were included in Study II. These subjects were also included in Studies III and IV, together with additional subjects, as more twins were recruited after the Study II. A total of 321 individuals (192 females and 129 males) were included in Study III. Data from 399 individuals (244 females and 155 males) were included in Study IV. Nearly equal amounts of MZ twins as DZ twins participated in each study. The participants were aged 21-24 years. Mean age of the participants was 22.6, 22.8, and 22.7 years in Studies II, III, and IV, respectively.

4.1.3 Australian twins (II-IV)

The Australian twins were derived from the Brisbane Adolescent Twin Studies project (Wright and Martin, 2004). Data from 199 twin individuals (107 females and 92 males) were included in Studies II and III. These subjects were also included in Study IV, together with additional participants, as more twins were recruited after Studies II and III. A total of 413 individuals (231 females and 182 males) were

included in Study IV. The data included more DZ twins than MZ twins in each study. The subjects were aged from 10 to 18 years. Mean age of the subjects was 14.3 years in Studies III and IV and 13.7 years in Study IV.

4.1.4 Danish twins (II-IV)

The Danish twins were derived from the Danish Twin Registry (Skytthe et al., 2006; Benyamin et al., 2007). A total of 336 twin individuals (178 females and 158 males) participated in Studies II-IV. Data were collected only from DZ twins. This subsample included 116 complete DZ twin pairs. The participants were aged from 24 to 60 years (mean age 44.9 years).

4.1.5 British twins (II, IV)

The twins from the United Kingdom were derived from the UK Adult Twin Registry (Spector and Williams, 2006). A total of 163 twin individuals (156 females and 7 males) participated in Studies II and IV. This subsample included 60 MZ and 20 DZ complete twin pairs. The age of the subjects ranged from 19 to 83 years (mean age 54.9 years).

4.2 Data collection

4.2.1 Procedure

The phenotypic data of the Finnish family study (I) were collected during the subjects' revisit to the clinic (in Helsinki) to participate in the Finnish migraine family study (in 2003-2004). Data from Finnish and Australian twins (II-IV) were collected during the participants' visit to twin research units in Helsinki and in Brisbane, respectively (in 2005-2007). The data from the UK twins were collected from volunteers in July 2006 at the twin gathering (TwinParty) in London. The data from Danish twins were collected by mail; materials were mailed to the subjects (in fall 2006), who performed the tasks at home and returned their responses by mail.

The study protocols and ethical principles applied in the study were approved by the appropriate local ethics committees and data protection agencies in the respective countries. All subjects gave their informed consent before participation.

4.2.2 Demographics and related information

The participants of the Finnish family study (I) were asked to complete a questionnaire regarding their sex, year of birth, and smoking habits in addition to other issues not considered in this study. The twin individuals (II-IV) filled out a questionnaire, providing information about sex and year of birth (in all countries), smoking habits (in Denmark, Finland, and UK), current cold and flu and other smelling hindrances (in Australia, Denmark, and Finland), and use of hormonal contraceptives (females in Finland and Denmark).

4.2.3 Self-ratings of olfactory function and odor annoyance

The subjects used seven categories to compare their olfactory function with "other people's sense of smell at my age in general." The ratings were verbally anchored at "Far worse" (Rating 1), "As good" (4), and "Far better" (7). Respectively, the subjects compared their subjective odor annoyance, caused by everyday ambient odors (e.g. smells of foods), with "other people at my age in general" using seven categories verbally anchored at "As far less disturbing" (Rating 1), "In the same way" (4), and "As far more disturbing" (7).

4.2.4 Responses to odor stimuli

Finnish family study (I)

The scratch-and-sniff odor stimuli (cinnamon, turpentine, lemon, smoke, chocolate, rose, paint thinner, banana, pineapple, gasoline, soap, and onion) of the B-SIT (Doty et al., 1996) was used in this study. The B-SIT originally included a multiple-choice odor identification tasks only. Rating scales for perceived intensity and pleasantness of the individual odor stimuli were added to the test for the purposes of this study. Perceived intensity of an odor was rated using five categories anchored as "No odor" (Rating 1), "Weak odor" (2), "Moderate odor" (3), "Fairly strong odor" (4), and "Very strong odor" (5). Pleasantness of an odor was rated using five categories anchored as "Very unpleasant" (Rating 1), "Fairly unpleasant" (2), "Neither pleasant nor unpleasant" (3), "Fairly pleasant" (4), and "Very pleasant" (5).

Twin studies (II-IV)

A new set of odor stimuli was designed to be used in the twin studies. The odors were selected based on the preliminary results of the family study (cinnamon and lemon), a literature survey (androstenone and isovaleric acid), and other considerations (chocolate and turpentine). These six odor stimuli (together with a

blank) were presented in a scratch-and-sniff form. The odorants for each odor stimulus were microencapsulated each on a separate sheet, which enabled randomizing the order of presentation. The odorants were manufactured by Quest International (presently Givaudan), except for androstenone, which was produced by Sigma-Aldrich. Chemical characteristics of the stimuli are shown in **Table 3**.

All stimuli were presented to the twins in Australia, Denmark, and Finland, but only androstenone, cinnamon, isovaleric acid, and lemon were used in the UK. In addition, vanilla and another blank stimulus were presented for training the evaluation procedure (except in the UK). Each sheet also contained the scales for ratings of perceived intensity and pleasantness and the multiple-choice identification task with 13 answer options (no odor, rose, lemon, vanilla, chocolate, cinnamon, onion, malt, smoke, turpentine, sweat, urine, or another odor) and space for free naming if the option "another odor" was selected. The sheets also contained brief instructions for the evaluation tasks. The instructions and scales were printed on the sheets in the appropriate languages (Danish, English, and Finnish). The sheets were printed by Kent Art Printers (Kent, UK). The print layouts of the sheets are shown in **Appendix A**.

Table 3. ***Odors stimuli and corresponding odorants used in the twin studies (II-IV).***

Odor	Odorant				
	Common name	Systematic name	CAS number[a]	Purity (%)	Pure/solution
Androstenone	Androstenone	5-α-androst-16-en-3-one	18339-16-7	high[b]	0.1% in diethyl phthalate
Cinnamon	Cinnamaldehyde	(E)-3-phenyl-2-propenal	14371-10-9	99.0	pure
Chocolate	Chocolate aroma	na	na	na	pure
Isovaleric acid	Isovaleric acid	3-methylbutanoic acid	503-74-2	99.5	10% in diethyl phthalate
Lemon	Citronellal	3,7-dimethyl-6-octenal	106-23-0	92.5	pure
Turpentine	Turpentine[c]	na	9005-90-7	na	pure
Vanilla[d]	Vanillin	4-hydroxy-3-methoxybenzaldehyde	121-33-5	99.8	10% in diethyl phthalate

[a] Identifiers for chemicals assigned by the Chemical Abstracts Service (CAS).
[b] Exact purity of the commercial product (Sigma-Aldrich product no. A8008) was not supplied by the manufacturer.
[c] Major constituent of turpentine is α-pinene (CAS number 80-56-8).
[d] Used only for training in the odor evaluation procedure.
na, not applicable.

Test-retest reliability of the responses to odor stimuli (**Table 4**) was determined using responses given by 26 unrelated Finnish individuals (17 females, 9 males; aged 20-60 years, mean age 30 years). They performed the odor identification task and rated the perceived intensity and pleasantness of the odors in two sessions spaced six days apart. The responses to androstenone were excluded from the odor identification score and the calculations of mean perceived intensity and pleasantness ratings (IV) because androstenone shows substantial specific anosmia (Bremner et al., 2003), and thus, may elicit a very dissimilar pattern of responses than the other odors studied.

Table 4. ***Test-retest reliability of responses to the odor stimuli used in the twin studies (II-IV).***

Response	Odor	Correlation coefficient		
		Pearson's r	Spearman's ρ	Intraclass correlation
Perceived intensity	Androstenone	0.84	0.80	0.84
	Chocolate	0.75	0.65	0.72
	Cinnamon	0.77	0.78	0.76
	Isovaleric acid	0.47	0.47	0.47
	Lemon	0.80	0.75	0.67
	Turpentine	0.39	0.42	0.37
Mean perceived intensity[a]		0.80	0.84	0.78
Pleasantness	Androstenone	0.56	0.51	0.57
	Chocolate	0.82	0.81	0.81
	Cinnamon	0.51	0.38	0.47
	Isovaleric acid	0.61	0.62	0.60
	Lemon	0.81	0.67	0.82
	Turpentine	0.51	0.43	0.52
Mean pleasantness[a]		0.78	0.64	0.76
Odor identification[a]		0.72	0.72	0.72

[a] Calculated based on responses to listed odors, except androstenone.

4.3 Data analysis

4.3.1 Basic statistical analysis (I-IV)

In the family study (I), Student's *t*-test, Mann-Whitney *U*-test, correlation analysis, and analysis of variance (ANOVA) were applied, where appropriate, using statistical software SPSS, versions 12 and 13. In the twin studies (II-IV), the statistical package Stata, version 8, was used in addition to SPSS. In these studies, the analyses based on individuals, regression analysis and Wald test, were corrected for the paired structure of the data (clustering in twin pairs) using the procedures for complex survey designs (svy) in Stata.

4.3.2 Heritability and linkage analysis of family data (I)

The variance components linkage analyses with the genome-wide marker data of 350 polymorphic microsatellite markers were performed using the program Merlin, version 1.0.1 (Abecasis et al., 2002). Merlin produced the heritability estimates jointly with the results of the linkage analysis. Statistical significance of the heritability estimates were evaluated using the QTDT program (Abecasis et al., 2000). Most genome-wide analyses were automated using AUTOGSCAN program (Hiekkalinna et al., 2005).

4.3.3 Genetic modeling of twin data (II-IV)

Quantitative genetic modeling of twin data was performed using the statistical package Mx, versions 1.5 and 1.7 (Neale et al., 2003). Within-pair intraclass correlations of MZ and DZ twins were calculated and used as a criterion for selecting an appropriate univariate model (ACE or ADE model) for the starting point of analyses (II-IV). The most parsimonious models were searched, fit of the models to the data were explored, and estimates of the relative contributions of the variance components to phenotypic variation were calculated (see Section 2.3.2). In Study II, a bivariate model (Cholesky decomposition) was employed to determine genetic and environmental correlations of two variables (perceived intensity and pleasantness of androstenone odor). In Study IV, a multivariate independent pathway model was applied to search for potential common genetic and environmental effects underlying the variation in intensity ratings of individual odors.

5 RESULTS

5.1 Identification of odors

Heritability estimates for odor identification were very low both in the family study (I) and in the twin study (IV). In the family study (12 stimuli of B-SIT), the heritability estimate for identification varied from 10% to 13% depending on the covariates used in the analysis. In the twin study (five stimuli), the estimate for additive genetic effects was 14% under the AE model (**Table 5**). No evidence for genetic linkage was found for this trait (I).

Females outperformed males in odor identification in the family study (I); females and males identified, on average, 10.4 and 9.8 (out of 12) odors, respectively ($p<0.01$). The sex-difference was not observed in the twin study (IV). The odor identification score did not (linearly) correlate with age in the family study (I). In the twin study (IV), the correlation was significantly nonzero but negligible ($r=0.12$). However, an inverse U-shaped relationship was observed for odor identification and age in the family study (I) as the middle-aged scored higher than the younger or the older subjects (**Figure 4**). Results from twin study IV revealed that nonsmokers identified more odors than regular smokers, but cold/flu, other smelling hindrances, or use of hormonal contraceptives had no association with odor identification.

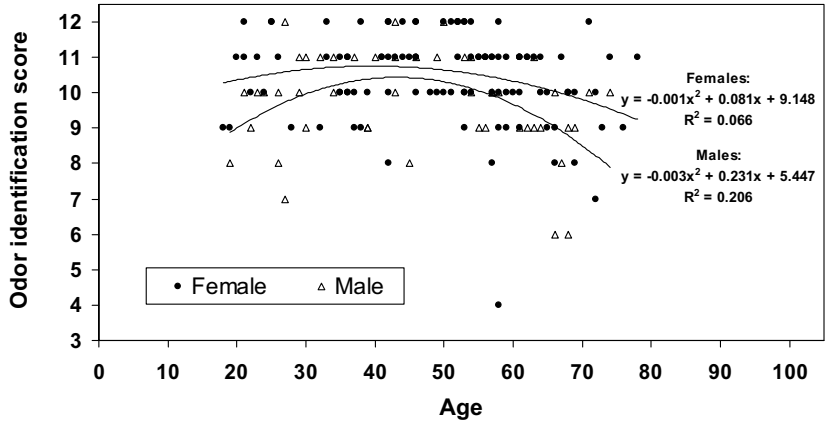

Figure 4. ***Odor identification score (B-SIT) by sex and age in the family study (note: scale for identification score starts from the chance level, 3). Second-order polynomial curves were fitted to data from females (upper curve) and males (lower curve) separately.***

Table 5. ***Results from genetic modeling of twin data for responses to odors (II-IV): within-pair intraclass correlations (ICC) and parameter estimates of variance components under the univariate AE model.***

Response	ICC		Parameter estimates (%)[a]	
	r(MZ)	r(DZ)	Additive genetic effects	Nonshared environmental effects
Perceived intensity				
-Androstenone	0.30	0.10	28 (13-41)*	72 (59-87)
-Chocolate	-0.09	0.04	0 (0-16)	100 (84-100)
-Cinnamon	0.17	0.12	22 (5-38)[#]	78 (62-95)
-Isovaleric acid	0.20	0.07	18 (1-34)	82 (66-99)
-Lemon	0.17	0.03	14 (0-27)	86 (73-100)
-Turpentine	0.04	0.08	11 (0-28)	89 (72-100)
-Mean[b]	0.24	0.10	23 (9-36)[#]	77 (64-91)
Pleasantness				
-Androstenone	0.25	0.07	21 (5-36)*	79 (64-95)
-Chocolate	0.05	0.12	12 (0-27)	88 (73-100)
-Cinnamon	0.19	0.10	21 (3-38)	79 (62-97)
-Isovaleric acid	0.02	0.13	14 (0-29)	86 (71-100)
-Lemon	-0.05	0.07	1 (0-15)	99 (85-100)
-Turpentine	0.10	0.12	15 (0-30)	85 (70-100)
-Mean[b]	0.19	0.06	16 (3-28)	84 (72-97)
Odor identification[b]	0.15	0.06	14 (1-27)	86 (73-99)
Self-rated olfactory function	0.12	0.14	16 (4-26)[#]	84 (73-96)
Self-rated odor annoyance	0.10	-0.03	5 (0-16)	95 (84-100)

[a] 95% confidence intervals in parentheses.
[b] Calculated based on responses to listed odors, except androstenone.
*, significant additive genetic component ($p < 0.05$).
[#], significant familiality.

5.2 Perceived intensity of odors

In the family study (I), ratings of perceived intensity of the odors showed the highest significant heritabilities for chocolate (31-34%, depending on covariates), rose (33-34%), and paint thinner (31%). In the twin study (II), heritability for the rated intensity of androstenone was 28% and 31% in the univariate (**Table 5**) and bivariate model (**Figure 5**), respectively. Suggestive linkage (LOD score 2.55) was found for the intensity of paint thinner on chromosome 2p14 (marker CHLC.GATA8F03.505 at position 91.23 cM) (I).

Perceived intensity and pleasantness of androstenone were moderately correlated (r=-0.27; phenotypic correlation) (II). A strong genetic correlation (r_g=-0.86) was observed between the ratings of intensity and pleasantness for androstenone; however, the environmental correlation was negligible. This suggests that the genetic correlation alone explains the phenotypic correlation. In addition, intensity and pleasantness had 74% of genetic variance in common (r_g^2), thus probably being influenced by an overlapping set of genes.

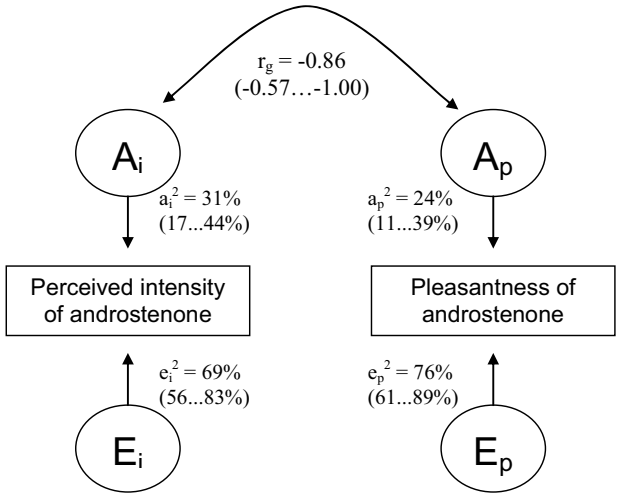

Figure 5. ***Relative contributions of additive genetic and nonshared environmental effects (a^2 and e^2, respectively) to variation in perceived intensity and pleasantness of androstenone odor and genetic correlation (r_g) between the traits according to the bivariate Cholesky decomposition (95% confidence intervals in parentheses).***

Females rated the intensity of odors in general as higher than males, in both the family study (I) and in the twin study (IV). For individual odors, females rated chocolate, rose, paint thinner, pineapple, gasoline, and soap (I) as well as androstenone (II) and turpentine (III) as more intense than did males.

A moderate negative correlation was observed between mean intensity ratings and age in both the family study (r=-0.26) and the twin study (r=-0.27). Smoking habits or other nongenetic factors were not associated with intensity ratings in any study. Several odors were evaluated as more intense by subjects who correctly identified them than by those who did not. Cinnamon and rose odors in the family study (**Figure 6**) and cinnamon, chocolate, and turpentine odors in the twin study (III) were rated as more intense when they were identified than when misidentified.

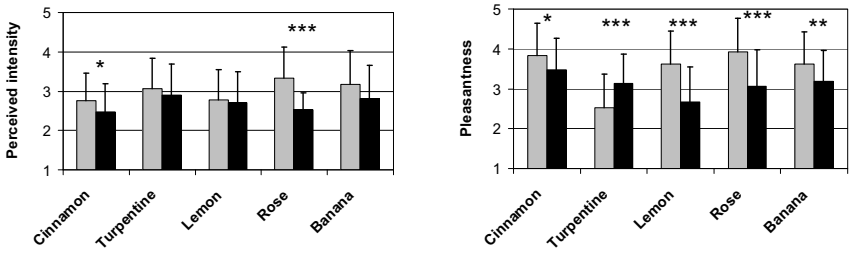

Figure 6. ***Average perceived intensity and pleasantness of odors by subjects who identified the respective odor correctly (gray bars) or incorrectly (black bars) in the family study. Odors that were identified correctly by more than 80% of the subjects were not included in the comparison (error bars denote standard deviation; *, p < 0.05, **, p < 0.01, ***, p < 0.001).***

5.3 Pleasantness of odors

Pleasantness ratings of cinnamon, lemon, and smoke showed the highest heritabilities (62-65%, 40-42%, and 40%, respectively) in the family study (I). In twin studies (II, III), androstenone exhibited the highest heritability; 21% and 24% in the univariate (**Table 5**) and bivariate models (**Figure 5**), respectively. The heritability estimate for pleasantness of cinnamon was 21%, albeit nonsignificant, in the twin study (III) (**Table 5**). Suggestive evidence of linkage (LOD score 3.01) was found for the pleasantness of cinnamon odor on chromosome 4q32.3 (marker AFM295YE5 at position 163.65 cM) in the family study (I). In addition, two other suggestive linkages were observed (LOD scores 2.10 and 2.56) for the pleasantness of cinnamon (**Figure 7**).

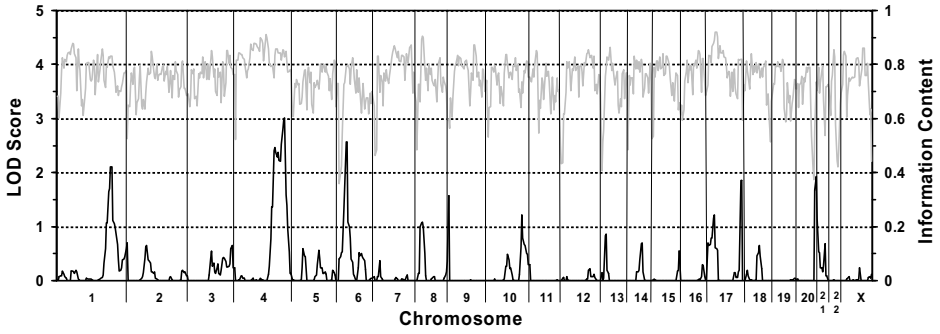

Figure 7. ***Genome-wide screen for loci linked to the pleasantness of cinnamon odor (black line, LOD scores for the markers; gray line, information content).***

Females rated rose odor as more pleasant and turpentine, paint thinner, and gasoline odors as less pleasant than males in the family study (I). The results for turpentine were replicated in the twin study (III). Smoking was associated with experienced pleasantness only in the case of chocolate (I) and isovaleric acid (III). In both the family (I) (**Figure 6**) and twin study (III), cinnamon odor was rated as more pleasant and turpentine odor as less pleasant by those who identified the respective odor correctly than by those who did not. In addition, lemon, rose, and banana odors in the family study (I) (**Figure 6**), as well as chocolate odor in a twin study (III) exhibited similar enhanced pleasantness when identified.

5.4 Self-ratings of olfactory function and odor annoyance

Self-rated olfactory function correlated moderately (r=0.30) with experienced odor annoyance. Neither of these self-ratings correlated with the odor identification score or age. Females and regular smokers rated odor annoyance as higher than did males and nonsmokers, respectively. Olfactory function was rated lower by subjects reportedly suffering from smelling hindrance (e.g., allergy, asthma, or nasal blockage) than by those who not reported such. Also, regular smokers rated their olfactory function as lower than nonsmokers (IV).

6 DISCUSSION

6.1 Olfactory function

6.1.1 Identification of odors

Olfactory function measured by odor identification ability appeared to be modified by environmental rather than genetic factors. Heritability estimates for odor identification were approximately the same size, but very low (10-14%) and nonsignificant in both the family (I) and twin study (IV).

Segal et al. (1995) found evidence for a genetic influence on odor identification in males, but not in females. Their findings were based on intraclass correlations of a relatively small number of twins and no heritability estimate was calculated. Finkel et al. (2001) observed significant heritability of 29% for odor identification. However, one of the six stimuli used in their study was androstenone, for which specific anosmia has been demonstrated (Bremner et al., 2003). This specific anosmia is genetically determined (Wysocki and Beauchamp, 1984). Thus, the heritable variation in androstenone perception may have contributed to their heritability estimate for (overall) odor identification.

As suggested by the very low heritability estimate, no genetic linkage was found for odor identification in the genome-wide linkage scan (I). However, Pinto et al. (2008) detected suggestive linkage for hyposmia (as measured by the same odor identification test used in Study I, the B-SIT) on chromosome 4q. They selected individuals with severe hyposmia without comorbidities, such as present cold, from a population of Hutterites in the USA. Their final sample included seven individuals who could be traced to the same very large pedigree, implying some sort of inheritance of hyposmia. The discrepancy between the results of the Study I and those of Pinto et al. (2008) may be explained by differences in study samples and in the trait examined; these authors focused on the hyposmic individuals, whereas Study I explored quantitative variation in odor identification in the general population. This quantitative variation may be polygenic and the relative contribution of each gene thus quite small. This would make it difficult to detect underlying genes using linkage analysis.

Females scored higher than males on the odor identification task in the family study (I). This superiority of females is in line with the results of numerous former studies (e.g. Doty et al., 1984; Wysocki and Gilbert, 1989; Murphy et al., 2002; Larsson et al., 2004). However, this sex difference was not observed in the twin study (IV).

This may partly be explained by the lower mean age of participants in the twin study (28.6 years) than in the family study (49.1 years). The sex difference in odor identification tends to be pronounced in the elderly, probably because the ability of males to identify odors starts to diminish at an earlier age than that of females (Wysocki and Gilbert, 1989; Brand and Millot, 2001).

An inverse U-shaped relationship was observed between odor identification score and age in the family study (I), as the middle-aged scored better than their younger or older counterparts. A similar curvilinear relationship was found by Doty et al. (1984b), Wysocki and Gilbert (1989), and Segal et al. (1995, in males). No clear linear correlation between odor identification and age was found. These observations imply that exposure to odors and learning can enhance odor identification ability until deterioration of olfaction outweights their effect in old age.

Subjects who identified an odor correctly tended to rate it as more intense and either more pleasant or more unpleasant than those who misidentified the odor (I, III). A strong, clearly perceived stimulus logically could result in higher intensity ratings and be easier to identify than a weaker stimulus. However, odor stimuli, especially those of the B-SIT used in the family study (I), appeared to be at a suprathreshold level for normosmics (mean intensity rating was 3.2 on a scale from 1 to 5). Thus, an association evoked by the name of the odor may be another factor contributing to the differences. For example, lemon, rose, and banana odors, which are likely to evoke positive associations, were rated as more pleasant by those who identified them correctly than by those who did not (I).

Odor identification score did not correlate with the self-rated olfactory function (IV). This result in consistent with several studies reporting a lack of agreement between self-reported and measured olfactory function (Landis et al., 2003; Philpott et al., 2006; Cameron, 2007; Pinto et al., 2008) and supports the notion that objective testing of the olfactory function cannot be replaced by self-ratings (Nordin et al., 1995; Murphy et al., 2002; Philpott et al., 2006; Pinto et al., 2007). The invisible role of the sense of smell in daily life (Landis et al., 2003; Gudziol et al., 2006) and the slow progress of olfactory impairments (Welge-Luessen et al., 2005) are likely causes for inaccurate subjective ratings of olfactory function. Although many elderly individuals and patients with diseases that have deleterious impact on olfaction are unaware of their smell deficit (Nordin et al., 1995; Murphy et al., 2002; Gudziol et al., 2007), individuals aware of the potential cause of olfactory dysfunction may, in turn, underestimate their sense of smell (Nordin et al., 1995). Likewise, in Study IV, olfactory function was rated as lower by subjects who reported potential chronic smelling hindrance (e.g. allergy, nasal blockage) than those who did not, but no difference in odor identification was found between the groups.

Self-rated olfactory function correlated moderately (r=0.30) with self-rated odor annoyance (IV). Thus, olfactory function may reflect odor annoyance rather than actual olfactory acuity. Neither of the self-ratings displayed significant heritability, underlining the role of individual experiences for these traits.

6.1.2 Perceived intensity of odors

Experienced intensity of androstenone odor showed a significant heritability of ~30% (II). Detection of androstenone odor was earlier found to be genetically determined (Wysocki and Beauchamp, 1984; Gross-Isseroff et al., 1992; Pause et al., 1998), and an underlying olfactory receptor gene polymorphism has recently been identified (Keller et al., 2007). However, the present estimate provides the first quantitative information about the overall contribution of genetic factors to sensitivity to androstenone.

Heritability estimates for perceived intensities in the family study (I) also were at ~30% at the highest (for chocolate, rose, and paint thinner). Suggestive evidence for linkage (LOD score 2.55) was found for the perceived intensity of paint thinner (I). Significant familiality (sum of genetic and shared environmental effects) was detected for experienced intensity of cinnamon odor in the twin study (III). Under the AE model, the relative contribution of additive genetic effects was 22%, but the genetic component was not significant. The heritability estimates for perceived intensity of the other odors were low and not insignificant (I-III). The heritability estimates calculated from family data (I) may be slight overestimates compared with those from twin data (II, III) because the influence of shared (common) environments cannot be ruled out in the former. This, together with differences in odor stimuli, may explain why heritability estimates in general, and specifically for the intensity of chocolate, were higher in the family study (I) than in the twin studies (II, III).

Females rated odors in general as more intense than males in both the family (I) and in the twin study (IV). Females also rated several individual odors as more intense than did males, but the reverse was not observed for any odor (I-III). These results support the notion that the olfactory acuity of females is superior to that of males, if suprathreshold ratings are assumed to reflect olfactory abilities (Doty and Laing, 2003) and if males and females used the rating scales similarly. In addition, mean perceived intensity of odors was negatively linearly correlated with age in both the family (I) and the twin study (IV). This result is consistent with the concept that the sense of smell declines with age (Rawson, 2006).

6.2 Odor hedonics

Pleasantness of cinnamon odor showed the highest heritability (65%) of the traits studied (I). However, this heritability estimate was based on family data, and thus, potential inclusion of shared environmental effects should be borne in mind. Not surprisingly, the strongest evidence for linkage in the study (LOD score 3.01) was found for the pleasantness of cinnamon, on chromosome 4q32.3. While this linkage did not reach genome-wide significance after correction for multiple testing, it provides suggestive evidence for genetic modification of odor hedonics. It is noteworthy that Pinto et al. (2008) also reported suggestive linkage on chromosome 4q, however, for hyposmia, a trait fairly different from the pleasantness of cinnamon. Nevertheless, both studies found tentative evidence for a genetic element modifying olfactory-related traits residing on chromosome 4q, where no functional olfactory receptor genes are known to exist (Malnic et al. 2004). Thus, interestingly, these results imply that chromosome 4q harbors a yet unknown genetic factor that modifies responses to odors. Although these results are, at present, only suggestive, every clue that could shed light on the issue and direct further research is valuable, as our understanding of relationships between genes and odor perception is still in its infancy.

Pleasantness of androstenone was found to be modestly but significantly heritable (21-24% depending on the model employed) in the twin study (II). Perceived intensity and pleasantness of androstenone odor correlated moderately (r=-0.27). A strong genetic correlation in the absence of an environmental correlation indicates that the phenotypic correlation between the traits can best be explained by the genetic correlation alone, and thus, the traits are influenced by an overlapping set of genes. This is supported by Keller et al. (2007) who demonstrated that polymorphism of odorant receptor gene *OR7D4* is associated with both perceived intensity and pleasantness of androstenone odor. The results of the present study (of cinnamon and androstenone) and those of Keller et al. (2007) are presumably the first evidence for the involvement of genetic effects in odor hedonics. However, genetic factors may influence odor pleasantness indirectly by modifying the way an odor is perceived rather than by directly influencing the pleasantness of shared perceptions.

Males rated turpentine, paint thinner, and gasoline odors as more pleasant and rose odor as less pleasant than females in the family study (I). The result for turpentine was replicated in a twin study (III). These sex-specific differences in hedonic ratings may reflect differences in associations evoked by the odors. Probably due to this sex-specific variability in preferences, the mean pleasantness did not differ between the sexes.

6.3 Methodological considerations

6.3.1 Subjects

The subjects were not recruited for this study alone, but also for various other clinical studies (I-IV). There should therefore be no selection biases resulting from recruitment. Moreover, the responses of the family sample and the twin samples in Finland and Australia were recorded in clinical environments together with other tests unrelated to odor evaluation. In the UK, the odor evaluations were collected from volunteers in a twin assembly; thus, the possibility exists that only the twins most interested in odors participated. Similarly, in Denmark, participation in the odor evaluation depended on the activity of the twins who received the testing material by mail. However, the most motivated individuals probably also produced the most reliable data, although individuals with reduced olfactory ability might have been less frequent among the respondents than among the refusers.

The data collection was designed to maximize the statistical power for genetic analyses. Thus, genetically as informative samples as possible were collected, but the data were not optimized for comparisons of sexes or populations from different countries, or for exploring the effects of age, smoking, or other nongenetic factors. Alhough the twin data were collected from four countries, comparisons between the populations were problematic since the samples from different countries were not matched for age and sex, and only four out of six stimuli was used in the UK.

6.3.2 Odor stimuli

Odor stimuli were presented on the scratch-and-sniff form. This provided a quick and easy way to administer the stimuli and enabled the collection of a large data set from four countries, including data collection by mail in Denmark.

In the family study (I), the stimuli in a commercially available smell identification test, B-SIT, were used. The test-retest reliability of the B-SIT is high ($r = 0.71$, Doty et al., 1995), normative data for interpreting the results are available, and the test is widely used for clinical and research purposes. However, the reliability of ratings of the perceived intensity and pleasantness was not determined for individual odors in the B-SIT. In the twin studies (II-IV), a set of tailor-made odor stimuli was used. The test-retest reliability of responses to the stimuli was reasonably high in most cases, but suboptimal for some traits (**Table 4**).

Measurement of olfactory function based on odor identification scores appears not to be highly dependent on the way the stimuli are presented, as long as the method produces

reliable data (Doty and Laing, 2003). Identification tests utilizing scratch-and-sniff stimuli, especially the UPSIT, are used very widely (Doty, 2001). Furthermore, former studies exploring genetic influences on odor identification (or hyposmia) have employed the scratch-and-sniff stimuli of the UPSIT (Segal et al., 1995), the B-SIT (Pinto et al., 2008), and the *National Geographic* smell test (Finkel et al., 2001).

Odor identification tests, such as the UPSIT and the B-SIT, are clearly adequate for clinical use to screen for olfactory deficits (Doty and Laing, 2003). How well odor identification tests measure olfactory acuity remains obscure. Odor identification tasks require ability to detect and recognize odors but also some degree of cognitive skills even when familiar odors and descriptors are used. Acquired odor naming skills may provide an advantage to middle-aged subjects, although their olfactory sensitivity may be no better than that of younger subjects. This is supported by the findings of the present study; the middle-aged scored best in odor identification in the family study (I), and there was a moderate negative correlation between subject's age and mean perceived intensity of odors in both the family (I) and the twin study (IV).

Pleasantness ratings of odors may not vary as systematically as intensity ratings (Distel and Hudson, 2001; Bensafi et al., 2007). If an odor evoked an association about the origin of the odor, the association probably influenced the evaluation of the odor's pleasantness. This was reflected in the dissimilar pleasantness ratings among subjects who identified an odor correctly (association likely) and those who did not (association unlikely).

6.3.3 Analyses

Most measured traits were regarded as continuous variables and analyzed using parametric methods, even when the variables contained only five categories. This may have introduced some uncertainty in the results. However, although the kurtosis of distributions of some variables was higher than optimal, most variables were roughly normally distributed. The underlying phenomena also were assumed to be distributed normally.

In the twin studies (II-IV), the size of the pooled sample was large compared samples in other similar studies (**Table 2**). However, as subjective responses were measured, the data inevitably included measurement error. This was reflected in rather low test-retest reliabilities of responses to some individual odors, although reliabilities for composite variables (e.g., mean perceived intensity) were high (**Table 4**). The error variation probably hindered the detection of genetic variation. Thus, the estimates of the genetic effects are likely to be conservative; in some cases, the genetic component may have been underestimated or gone undetected altogether.

7 CONCLUSIONS

Genetic variation was noted for responses to specific odors. A moderate genetic contribution to the perceived intensity and pleasantness of androstenone odor was observed. A suggestive evidence for the locus underlying the heritability of pleasantness of cinnamon odor was found. This study was among the first to provide evidence for genetic contribution not only to perceived intensity but also to pleasantness of individual odors.

In contrast, genetic variation appeared not to influence general olfactory function. Moreover, phenotypic variation in perceived intensity and pleasantness of most odors studied was only modestly influenced by genetic effects. However, measurement of human responses to odors is challenging due to the inevitable subjectivity of most responses. Consequently, the phenotypic variation measured can be assumed to include error variation, which, in turn, may have hindered detection of variation due to genetic factors. Some genetic effects may have thus been underestimated and the results may be rather conservative.

The olfactory system relies upon combinatorial coding for odor recognition, and thus, several olfactory receptor genes may be involved in the recognition of one odor. The relative contribution of each receptor gene is, however, likely to be small and to go undetected in linkage analysis. In addition, this study reinforced the notion that responses to odors can also be influenced by genes other than olfactory receptor genes.

This was the first study to employ quantitative genetic modeling of twin data to estimate heritability of responses to individual odors. In addition, a genome-wide linkage screen was used to search for underlying genetic loci for the first time for responses to individual odors. Furthermore, this study was among the first studies to explore the genetic background of olfaction using psychophysical testing of human subjects and modern genetic analyses. This type of study can provide data that are more applicable for practical use than studies employing only animal or cell models.

The study provided information about the relative contributions of genetic factors to responses to odors. This information is valuable when evaluating whether a rationale exists for a more laborious search for underlying genes and when estimating the magnitude of genetic control over odor perception and preference, which are essential factors that modify food choice. Based on this study, further research on responses to odorants showing specific anosmia, interaction between odor exposure and expression of the OR genes, and genetic contribution to electrophysiologically measured responses to odors might be advantageous.

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Helsinki, June 6th, 2008

Antti Knaapila

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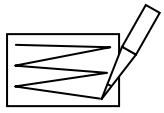
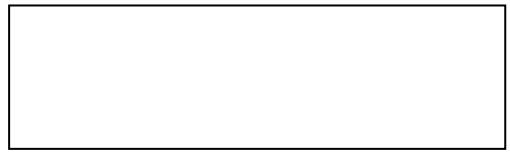
APPENDIX A: ODOR SHEETS USED IN STUDIES II-IV

ENG 093

Instructions: Please use a pencil to scratch the label from side to side five times (see picture). Immediately after that, sniff the label from close distance. Rate pleasantness and intensity of the odour first, then try to identify the odour. For pleasantness and intensity ratings, circle any number from 1 to 9 that corresponds best to your opinion.





Task 1. Rate the pleasantness of the odour.

Extremely unpleasant				**Neither pleasant nor unpleasant**				**Extremely pleasant**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

Task 2. Rate the intensity of the odour.

No odour								**Extremely strong odour**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

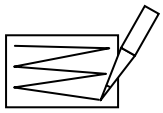
Task 3. Tick an alternative that best describes the odour.

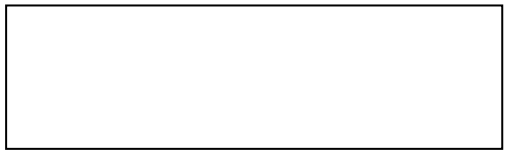
☐ No odour	☐ Chocolate	☐ Smoke
☐ Rose	☐ Cinnamon	☐ Turpentine
☐ Lemon	☐ Onion	☐ Sweat
☐ Vanilla	☐ Malt	☐ Urine

☐ Another odour (please describe): _____

DK 093

Vejledning: De bedes bruge en blyant til at skrabe fra den ene side til den anden side fem gange på seddelen (se tegning). Lugt til seddelen på kort afstand umiddelbart efter. De bedes vurdere lugtens behagelighed og styrke først og dernæst prøve at identificere lugten. Ved vurdering af behagelighed og styrke bedes De tegne en cirkel omkring det tal fra 1 til 9, som svarer bedst til Deres mening.





Opgave 1. Vurdering af lugtens behagelighed.

Yderst ubehagelig			**Hverken behagelig eller ubehagelig**					**Yderst behagelig**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

Opgave 2. Vurdering af lugtens styrke.

Ingen lugt								**Yderst kraftig lugt**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

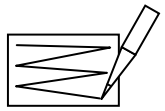
Opgave 3. De bedes krydse af ved den mulighed, som bedst beskriver lugten.

☐ Ingen lugt	☐ Chocolade	☐ Røg
☐ Rose	☐ Kanel	☐ Terpentin
☐ Citron	☐ Løg	☐ Sved
☐ Vanille	☐ Malt	☐ Urin

☐ En anden lugt (De bedes beskrive den): _____

FIN 093

Ohje: Raaputtakaa raaputuspintaa lyijykynällä laidasta toiseen viisi kertaa (katsokaa kuvaa). Välittömästi sen jälkeen haistakaa pintaa läheltä. Arvioikaa ensin hajun miellyttävyys ja voimakkuus, ja yrittäkää sitten tunnistaa haju. Hajun miellyttävyyden ja voimakkuuden tapauksessa ympyröikää asteikolta 1-9 se numero, joka parhaiten vastaa arviotanne.



Tehtävä 1. Arvioikaa hajun miellyttävyys.

Äärimmäisen epämiel-lyttävä				**Ei miellyttävä eikä epämiel-lyttävä**				**Äärimmäisen miellyt-tävä**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

Tehtävä 2. Arvioikaa hajun voimakkuus.

Ei hajua								**Äärimmäisen voimakas haju**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

Tehtävä 3. Rastittakaa vaihtoehto, joka parhaiten kuvaa hajua.

☐ Ei hajua	☐ Suklaa	☐ Savu
☐ Ruusu	☐ Kaneli	☐ Tärpätti
☐ Sitruuna	☐ Sipuli	☐ Hiki
☐ Vanilja	☐ Mallas	☐ Virtsa

☐ Muu haju (kuvailkaa): _____

APPENDIX B: ORIGINAL PUBLICATIONS